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Photoreaction of Psoralen and other Furocoumarins with Nucleic Acids

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The 360-nm photoinitiated reactions of certain furo[3,2-g]coumarins with DNA have been examined using ethidium fluorescence assays. Psoralen at 1.85×10^{-4} *M* gives 3.3×10^{-5}, 1.8×10^{-5}, and 4.5×10^{-6} interstrand cross-links/nucleotide with DNAs of (A + T) content 70, 60, and 50%, respectively. The relative rates of cross-linking of λ-DNA are 4-methylpsoralen > psoralen > angelicin ≫ 4-phenylpsoralen. Angelicin (isopsoralen) gives a small (12–14%) but reproducible amount of DNA interstrand cross-links. Addition of netropsin, an antibiotic that binds preferentially to (A + T)-rich regions, to *Clostridium perfringens* DNA reduces the extent of cross-linking by psoralen from 66 to 10% in 50 min. In contrast, pretreatment of DNA with olivomycin or chromomycin A_3 [which bind to (G + C)-rich regions] has little effect on psoralen cross-linking. Relative rates of monoadduction of furocoumarins to PM2-CCC-DNA detected by thermal depyrimidation and alkaline strand scission is angelicin > 4-methyl-4′,5′-dihydropsoralen > 4′,5′-dihydropsoralen ≳ 3,4-dihydropsoralen (no monoadduction), indicating angelicin is suitable for photolabeling of chromatin. Binding of netropsin to the PM2-DNA prevents cross-linking by angelicin but permits and enhances monoadduction. In contrast neither olivomycin nor chromomycin affects the reaction of angelicin with DNA. In the frozen solution, where the photoinduced cross-linking of DNA by psoralen may be suppressed, psoralen forms monoadducts about twice as readily as angelicin. Subsequent 360-nm irradiation of the psoralen monoadducts at ambient temperatures (and in separate experiments after dialysis to remove unreacted psoralen) completes the cross-links.

INTRODUCTION

Furo[3,2-g]coumarins,[1] including psoralen (1a) (Fig. 1), 8-methoxypsoralen, 5-methoxypsoralen, and 4,5′,8-trimethylpsoralen exhibit photosensitizing properties on viruses (*1*, *2*), bacterial cells (*3–5*), and mammalian cell cultures (*6*, *7*) upon irradiation with near (320–380 nm) light. For example, they cause lethal and mutagenic effects on bacterial cells (*3–5*), inactivate DNA viruses (*2*). and cause destruction of the tumor-producing capacity of Ehrlich ascites tumor cells in mice (*8*). Furocoumarins are used clinically to treat skin-pigmentation disorders such as vitiligo in the phototherapy of psoriasis, and to increase tolerance of sensitive skin to sunlight (*9*).

Furocoumarins photoreact directly with vital biological macromolecules, binding primarily to DNA and to a lesser extent to RNA and to proteins (*10*, *11*). The photochemical reaction appears to involve cycloaddition to the 5,6-bond of pyrimidines on the DNA, causing monoadduction as well as covalent cross-linking of the complementary strands of the DNA (*12–14*). Recently furocoumarins have been employed as

[1] The numbering system of the furocoumarins employed in this paper accords with general usage in this field, although it does not correspond strictly to IUPAC nomenclature rules.

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photochemical probes for chromatin structure in conjunction with denaturation microscopy (*15–17*).

The main advantage of these compounds is their ability to penetrate cells and viruses *in vivo* with no apparent disruption of cellular processes in the absence of photoexcitation (*15*). Furocoumarins show high specificity for DNA compared with proteins and form stable interstrand links with a high reaction efficiency without the accompanying degradation of DNA that is found with other cross-linking agents (*17, 26*).

Selective photochemically induced cross-linking of DNA would be very useful in preventing branch migration (*18*) in the study of mechanisms of DNA replication. Since

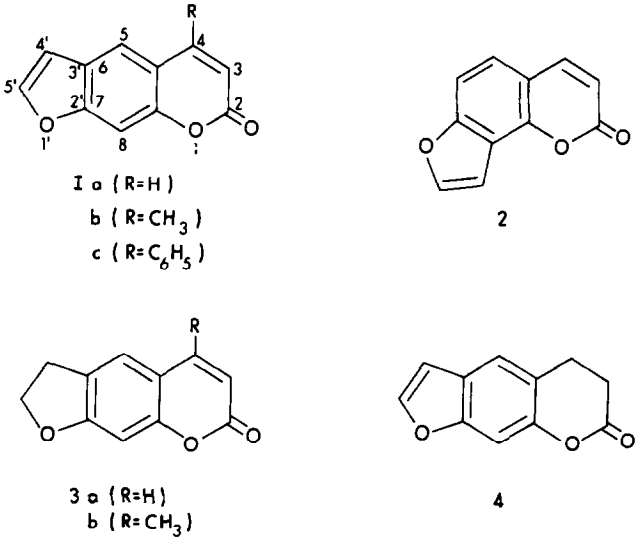

FIG. 1. Structural formulas for psoralen, **1a**; 4-methylpsoralen, **1b**; 4-phenylpsoralen, **1c**; angelicin, **2**; 4′,5′-dihydropsoralen, **3a**; 4-methyl-4′,5′-dihydropsoralen, **3b**; and 3,4-dihydropsoralen **4**.

this promising technique complements other approaches such as Q-banding in the investigation of chromatin structure (*19, 20*), more information was needed on the chemical modes of interaction of furocoumarins with nucleic acids. The possible pertubation of chromatin structure by the psoralen cross-linking probe itself, for example, is unknown (*16*).

Since fluorescence assays based on the intercalative dye ethidium bromide have high sensitivity (*21–23*), they are convenient for examining the detailed and various modes of interaction of the furocoumarins with nucleic acids. We report results from a study of this aspect of furocoumarin reactivity including the dependence on DNA base specificity, structural and stereochemical constraints on monoadduction and cross-linking of DNAs, and thermodynamic factors controlling the reactions. Experiments with DNA base-selective binding by netropsin, olivomycin, and chromomycin and their effects on psoralen cross-linking suggests ways in which the application of psoralen to chromatin mapping may be refined.

EXPERIMENTAL

Materials

Psoralen (furo[3,2-g]coumarin, **1a**) and angelicin (isopsoralen, furo[2,3-h]coumarin, **2**) were obtained from Dr. M. J. Ashwood-Smith. 4-Methylpsoralen (4-methylfuro[3,2-g]coumarin, **1b**), 4-methyl-4′,5′-dihydropsoralen (4-methyl-4′,5′-dihydrofuro[3,2-g]-coumarin, **3b**), 4′,5′-dihydropsoralen (4′,5′-dihydrofuro[3,2-g]coumarin, **3a**), 3,4-dihydropsoralen (3,4-dihydrofuro[3,2-g]coumarin, **4**), and 4-phenylpsoralen (4-phenyl-furo[3,2-g]coumarin, **1c**) were synthesized by literature procedures (*24*).

Olivomycin and chromomycin A_3 were obtained from Calbiochem. Netropsin was obtained from American Cyanamid, Lederle Laboratories. Ethidium bromide and calf thymus DNA were purchased from Sigma Chemical Co. The λ and PM2-CCC-DNAs[2] were prepared as before (*21*). *Escherichia coli* and *Clostridium perfringens* DNAs were obtained from Dr. A. R. Morgan.

Methods

Absorption spectra were measured in Spectrograde solvents and were run on a Beckman DB spectrophotometer or on a Gilford Model 250 spectrophotometer.

Fluorescence assay for detecting CLC sequences photochemically induced in DNA by furocoumarins. All measurements were performed on a G. K. Turner and Associates Model 430 spectrofluorometer equipped with a cooling fan to minimize fluctuations in the xenon lamp source. Wavelength calibration was performed as described in the manual for the instrument. One-centimeter-square cuvettes were used. The excitation wavelength was 525 nm, and the emission wavelength was 600 nm. The 100× scale of medium senstitivity was generally used, and water was circulated between the cell compartment and a thermally regulated bath at 22°C. Reactions were performed at 25°C in a total volume of 250 μl buffered with potassium phosphate at pH 7.0. Reaction solutions had concentrations of 0.5–1.6 A_{260} units of λ-DNA, 1.85×10^{-4} *M* furo[3,2-g]coumarin, and 2% ethanol. The solution was irradiated with a Sylvania black light at 360 nm at an intensity of 17.50 ergs/mm^2/sec [determined by means of 0.15 *M* potassium ferrioxalate chemical actinometer (*25*)]. Aliquots of 25 μl were removed at intervals and analyzed for the extent of cross-linking by addition to 2 ml of the standard pH 11.8 assay solution. This contains 20 m*M* potassium phosphate, pH 11.8, 0.2 m*M* EDTA, and 0.5 μg/ml of ethidium bromide. The fluorescence was measured using a blank without added sample. For the heat denaturation, the solutions were heated for 3 min at 96°C on a Temp-Blok and cooled to 23°C. Under these conditions only CLC-DNA shows return of fluorescence since the covalent links provide a nucleation site for renaturation (*22*, *23*). The ratio of the fluorescence after heating to the fluorescence before heating gave the extent of covalent cross-linking of the DNA. The concentrations of other components are indicated in the legends to the figures.

Dependence of efficiency of covalent cross-linking of DNA by furocoumarins on the (A + T) content of the DNA. The DNAs used were *Clostridium perfringens* [MW = 11.4×10^6; (A + T) content = 70%], calf thymus [MW = 9.1×10^6, (A + T) content = 60%], and *E. coli* [MW = 14.8×10^6, (A + T) content = 50%]. The molecular weights

[2] Abbreviations used: CLC, covalently linked complementary; CCC, covalently closed circular; OC, open circular; EDTA, ethylenediaminetetraacetic acid, disodium salt; A_{260}, absorbance at 260 nm.

were determined by sedimentation velocity studies. Allowance was made for differences in the average molecular weights. Since only one cross-link per DNA molecule is sufficient to produce rapid renaturation after cooling, DNAs of lower molecular weight require more cross-linking events per nucleotide residue to produce the same percentage of cross-linked DNA. Assuming a Poisson distribution of the links and further, that one link is sufficient to permit spontaneous renaturation of the molecule, the average number of cross-links per DNA molecule, m ($m = \ln(1/P_0)$, where P_0 is the proportion of unlinked molecules), was computed (*25*). The photochemically induced CLC sequences in the DNAs produced by the furocoumarins were detected by the experimental procedures described above.

Procedure for the determination of monoadduction of furocoumarins and dihydrofurocoumarins to PM2-CCC-DNA. Reactions were performed at 23°C in a total volume of 250 μl buffered at pH 7.0. Reaction solutions contained 1.0 A_{260} units of PM2-CCC-DNA (88% CCC), 1.85×10^{-4} *M* furocoumarin or dihydrofurocoumarin, 40 m*M* potassium phosphate, pH 7.0, and 2% ethanol. The solutions were irradiated with a Sylvania black light at 360 nm at an intensity of 17.5 ergs/mm^2/sec. Aliquots (25 μl) were removed at intervals and analyzed by adding to 2 ml of the standard ethidium fluorescence assay at pH 11.8. In a control it was shown that none of the components interfered with the ethidium fluorescence. Under these conditions unreacted PM2-CCC-DNA returns to register after heat denaturation because of topological constraints (*27*). Furocoumarin-adducted PM2-CCC-DNA suffers slow thermally induced depyrimidation at the site of addition. The exposed depyrimidated site is cleaved rapidly by the strong base and consequently shows a fall in fluorescence after denaturation (*28*). The ratio of the decrease in fluorescence (after heat denaturation and rapid cooling) to that of the control is a measure of the extent of alkylation.

Stepwise photoinduced cross-linking of DNA by furocoumarins. Reactions were performed at 23°C in a total volume of 500 μl buffered at pH 7.0. Reaction solutions had concentrations of 1.0 A_{260} of λ-phage DNA, 1.85×10^{-4} *M* furocoumarin, 40 m*M* potassium phosphate, and 2% ethanol. The solutions were irradiated at an intensity of 17.5 ergs/mm^2/sec for 10–20 min. The extent of cross-linking was determined by the CLC fluorescence assay. The solution was then transferred to a dialysis tube and dialyzed against pH 7.0 buffer (4 × 250 ml) for 48 hr to remove unattached psoralen. A nonirradiated control solution containing the same components was dialyzed in the same manner. The extents of cross-linking in the dialyzed DNAs were then analyzed by the fluorescence assay and were found to remain unchanged during the course of the dialysis.

Both the partially cross-linked DNA solution and the control solution were then irradiated at 360 nm for 30–60 min. No significant cross-linking of DNA was observed in the control solution.

Effect of DNA base-specific binding antibiotics on the psoralen-induced cross-linking of C. perfringens DNA. Reactions were performed at 23°C in a total volume of 250 μl buffered at pH 7.0. Reaction solutions had concentrations of 0.8 A_{260} of *C. perfringens* DNA, 1.85×10^{-4} *M* of psoralen, 0.04 *M* potassium phosphate, and 2–6% ethanol. Additional components were (a) 9.2×10^{-5} *M* netropsin, (b) 3.3×10^{-5} *M* chromomycin and 3.3×10^{-5} *M* $MgCl_2$, and (c) 3.3×10^{-5} *M* olivomycin, and 3.3×10^{-5} *M* $MgCl_2$.

The solutions were irradiated at an intensity of 17.5 ergs/mm^2/sec at 360 nm, and aliquots were withdrawn at intervals. Netropsin at 9.2×10^{-5} *M* shows zero absorption at 360 nm. Olivomycin and chromomycin at 3.3×10^{-5} *M* show an absorbance of 0.13 at 360 nm but do not detectably alter the rate of photoinduced cross-linking of DNA by psoralen. The extents of cross-linking were determined by the CLC fluorescence assay as described above.

Effect of DNA base-specific binding antibiotics on the psoralen- or angelicin-induced addition to PM2-CCC-DNA. Reactions were performed at 23°C in a total volume of 250 μl buffered at pH 7.0. Reaction solutions had concentrations of 1.2 A_{260} of PM2-DNA, 1.85×10^{-4} *M* of psoralen, 0.04 *M* potassium phosphate, and 2–5% ethanol. Additional components were (a) 9.2×10^{-5} *M* netropsin and (b) 3.3×10^{-5} *M* chromomycin and 3.3×10^{-5} *M* $MgCl_2$.

The solutions were irradiated at an intensity of 17.5 ergs/mm^2/sec, and aliquots were withdrawn at intervals and analyzed for alkylation as described above.

RESULTS AND DISCUSSION

Since furocoumarins are thought to interact preferentially with thymine residues of DNA (*12*), it follows that a correlation should exist between the efficiency of 360-nm light-induced covalent cross-linking and the (A + T) content of natural DNAs of different composition. Psoralen at a concentration of 1.85×10^{-4} *M* gives 3.3×10^{-5}, 1.8×10^{-5}, and 4.5×10^{-6} cross-links/nucleotide with DNAs of (A + T) contents of 70, 60, and 50%, respectively.

The photochemical reaction involved appears to be an allowed [π2s + π2s] cycloaddition (*29*) to the 5,6-bond of thymine residue on the DNA. The HOMO(π^*) of the photoexcited psoralen overlaps with the LUMO(π^*) of the ground state pyrimidine in a symmetry-allowed process. Photoaddition products involving the 4′,5′-furan double bond of psoralen with thymine bases have been isolated (*30*).

Using λ-DNA the relative rates of cross-linking of different furocoumarins under comparable conditions are (Fig. 2) 4-methylpsoralen > psoralen > angelicin. The result for angelicin (isopsoralen) indicates slow DNA cross-linking, reproducibly to the extent of 12–14% over 1 hr of irradiation. 4-Phenylpsoralen (4-phenylfuro[3,2-g]coumarin) fails to cross-link DNA owing to a competing and preferential photodimer formation. 4-Methyl-4′,5′-dihydropsoralen, 4′,5′-dihydropsoralen, and 3,4-dihydropsoralen do not cross-link DNA.

Incubation of λ-DNA with 9.2×10^{-5} *M* netropsin [an antibiotic that binds preferentially to XTTTX sequences, i.e., (A + T)-rich regions in the minor groove of bihelical DNA (*31*, *32*)] before irradiation at 360 nm in the presence of psoralen resulted in substantial reduction in the extent of cross-links produced (10% after 50 min) compared with the control (66% cross-links, Fig. 2).

In complementary experiments it was shown that neither chromomycin A_3 nor olivomycin [antibiotics which in the presence of Mg^{2+} bind strongly and preferentially to (G + C)-rich regions in the minor groove of duplex DNA (*33*, *34*)] has appreciable effect on the rate of photoinduced covalent cross-linking of λ-DNA by psoralen. Evidently binding of these antibiotics to the DNA duplex still leaves sufficient stretches

of (A + T)-rich areas exposed to permit intercalation by psoralen and substantial photo-induced interstrand cross-linking (Fig. 2).

In control experiments it was shown that neither netropsin, chromomycin A_3, nor olivomycin reacts photochemically with DNA at a wavelength of 360 nm. Netropsin shows zero absorption, and the other antibiotics show minimal absorption at 360 nm. Netropsin modifies the fluorescence behavior of the ethidium-PM2-DNA complex in a predictable manner, causing some decrease before heat denaturation by preferential binding but no displacement after heat denaturation and cooling when the antibiotic is destroyed and the DNA returns to register.

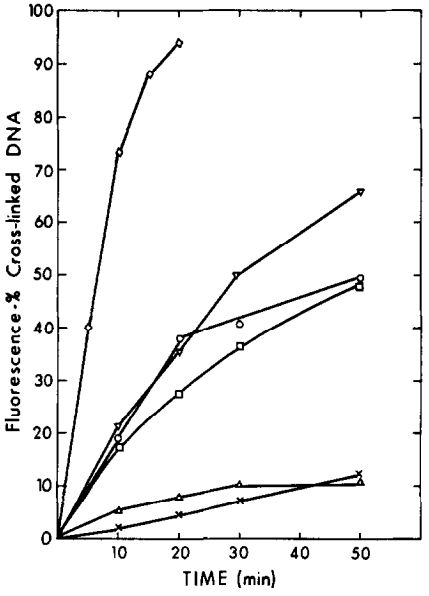

FIG. 2. Photoinduced cross-linking of λ-DNA with furo[3,2-g]coumarins and in the presence of DNA base-specific binding antibiotics. Reactions were performed at 25°C in a volume of 250 μl buffered at pH 7.0 with 40 m*M* potassium phosphate, contained 0.5–1.6 A_{260} units of λ-DNA and 1.85 × 10^{-4} *M* furo-[3,2-g]coumarin, and were irradiated at 360 nm at an intensity of 17.5 ergs/mm²/sec. Fluorescence readings are those after the heat denaturation and cooling cycle. ◊—◊, 4-methylpsoralen; ▽—▽, psoralen; △—△, psoralen and 9.2 × 10^{-5} *M* netropsin; □—□, psoralen, 3.3 × 10^{-5} *M* chromomycin, and 3.3 × 10^{-5} *M* $MgCl_2$; O—O psoralen, 3.3 × 10^{-5} *M* olivomycin, and 3.3 × 10^{-5} *M* $MgCl_2$; ×—×, angelicin.

In experiments with PM2-CCC-DNA, 9.2 × 10^{-5} *M* netropsin suppressed the photo-induced cross-links produced by psoralen but permitted monoadduction (see below). Chromomycin had no detectable effect on the psoralen cross-linking reaction (Fig. 3). Such experiments with selected antibiotics promise to be useful in probing chromatin structure in conjunction with existing methods of denaturation mapping employing psoralen. Additionally, since relatively little is known about the mode of action of netropsin against Rauscher virus-induced lymphoid leukemia in mice (*3*, *4*), the effects of psoralen and other cross-linking probes may prove useful in the understanding of the precise interaction of netropsin (and the related distamycin) with DNA.

Modified compounds of the coumarin class, like angelicin and 4,5-dihydropsoralen, that lack the capacity for light-induced cross-linking may nevertheless prove useful for

photoinduced labeling of chromatin when covalent cross-linking of strands is undesirable. A sensitive test for monoadduction is afforded by the use of PM2-CCC-DNA. Alkylation or photoinduced monoadduction of the latter can be detected by heat-induced depyrimidation and subsequent strand scission of the ribose–phosphate chain in the alkaline pH 11.8 ethidium bromide assay medium with consequent loss of potential intercalation sites for the ethidium so that the fluorescence eventually falls to zero. For relatively high levels of monoadduction there is proportionate loss of potential intercalation sites for ethidium, e.g., by steric hindrance, so that the fluorescence value falls (*28*). The thermally induced depurination process seems to be more efficient for

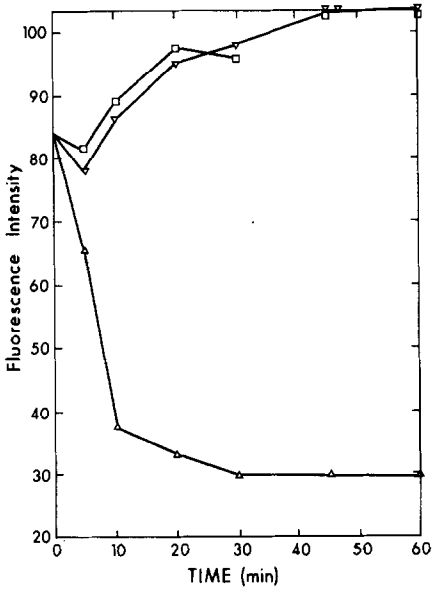

FIG. 3. Photoinitiated cross-linking of PM2-CCC-DNA with psoralen in the presence of DNA base-specific binding antibiotics. Reactions were performed at 23°C in 250 μl buffered at pH 7.0 with 40 m*M* potassium phosphate, contained 1.2 A_{260} units of PM2-CCC-DNA (84% CCC) and 1.85 $\times$ 10^{-4} *M* psoralen, and were irradiated at 360 nm at an intensity of 17.5 ergs/mm^2/sec. Additional components were: ▽—▽, none; □—□, 3.3 $\times$ 10^{-5} *M* chromomycin and 3.3 $\times$ 10^{-5} *M* $MgCl_2$; and △—△, 9.2 $\times$ 10^{-5} *M* netropsin.

charged alkylated DNA than for the corresponding neutral cyclobutane photoadducts described here.

Figure 4 shows that 1.84 $\times$ 10^{-4} *M* angelicin readily forms monoadducts and results in 20% heat-induced cleavage within 30 min. After this initial period a gradual return of fluorescence is indicative of slow covalent cross-linking, in accord with the previous results obtained from λ-DNA. Prior incubation of the PM2-CCC-DNA with netropsin completely suppresses the cross-linking and permits, or even enhances, the rate and extent of monoadduction. This result parallels that observed with psoralen. Under comparable experimental conditions with PM2-CCC-DNA, 4-methyl-4′,5′-dihydro-furo[3,2-g]coumarin (**3b**) and 5,5′-dihydropsoralen (**3a**) effect monoadduction and subsequent thermally induced DNA cleavage of 27 and 13% in 100 min, respectively. In contrast 3,4-dihydropsoralen (**4**) showed no evidence of photoinduced adduction with DNA. These findings support the contention that in the cross-linking of DNA by

psoralen it is the 4',5'-double bond of the furo[3,2-g]coumarin system which first reacts with thymine (*30*, *35*) and also indicates that angelicin would be the compound of choice for photolabeling. Photochemically produced interstrand cross-linking by psoralen is, as expected, accompanied by the more common monoadduction events. A comparison of the relative rates of formation of DNA monoadducts between psoralen and angelicin is possible if conditions are selected to suppress the completion of interstrand cross-links by the former. Irradiation of λ-DNA in the presence of the furocoumarins with 360-nm light in the frozen state was found to suppress the covalent

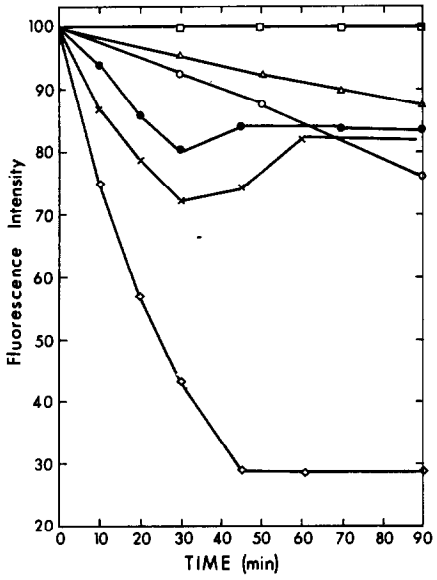

FIG. 4. Photoinitiated monoadduction followed by heat-induced depurination and alkaline strand scission of PM2-CCC-DNA. Reactions were performed at 23°C in 250 μl buffered at pH 7.0 with 40 m*M* potassium phosphate with 1.0 A_{260} unit of PM2-CCC-DNA (88% CCC), 1.85 × 10^{-4} *M* furo[3,2-g]-coumarin or dihydrofuro[3,2-g]coumarin and irradiation at 360 nm at an intensity of 17.5 ergs/mm²/sec. Fluorescence readings are those after the heat denaturation and cooling cycle. △—△, 4',5'-dihydropsoralen; ○—○, 4-methyl-4',5'-dihydropsoralen; ×—×, angelicin; □—□, 3,4-dihydropsoralen; ◇—◇, angelicin and 9.2 × 10^{-5} *M* netropsin; ●—●, angelicin, 3.3 × 10^{-5} *M* chromomycin, and 3.3 × 10^{-5} *M* $MgCl_2$.

cross-linking by psoralen observed at ambient temperatures by introducing conformational constraints on the DNA.

Figure 5 shows that the rate of monoadduct formation by psoralen under these conditions was about twice as fast as that by angelicin. No cross-linking is observed with psoralen under these conditions, since this would have resulted in an increase in the fluorescence values as observed in the CLC assays above.

By contrast when, after 50 min of irradiation of the frozen solution, the reaction mixtures were allowed to reach ambient temperature and then were irradiated with 360-nm light, covalent cross-linking occurred with psoralen, returning to a value of 80% renaturable PM2-DNA from a minimum of 38%. Very little cross-linking occurs with angelicin under these conditions. A control experiment was performed to demonstrate

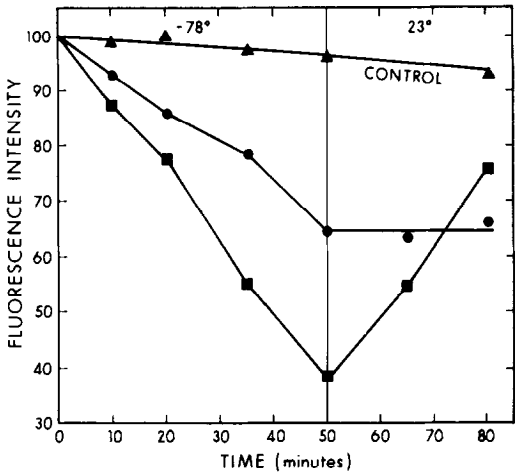

FIG. 5. Suppression of photoinduced covalent cross-linking of PM2-DNA by psoralen and comparison of relative rates of monoadduction by psoralen and angelicin. Reactions were performed at −78°C in a total volume of 250 μl buffered at pH 7.0 with 1 A_{260} unit of PM2-CCC-DNA, 40 m*M* potassium phosphate, 2% ethanol, and 1.85 × 10^{-4} *M* furo[3,2-g]coumarin and irradiation at 360 nm. ●—●, angelicin; ■—■, psoralen; ▲—▲ control. After 50 min of irradiation, the reactions were stopped and allowed to reach ambient temperature, and the irradiation was continued. Fluorescence values are those after the heat denaturation and cooling cycle.

that subsequent irradiation at room temperature completes cross-links from existing monoadducts and not due to photoexcitation of fresh psoralen molecules. Irradiation of λ-DNA at 360 nm at pH 7 in the presence of 1.84 × 10^{-4} *M* psoralen for 10- and 20-min periods gave 20 and 32% cross-linking, respectively. The solutions were dialyzed against pH 7 buffer to remove unattached psoralen and psoralen–photodimers. The extents of cross-linking in the dialyzed DNAs were shown by the CLC assay not to have changed during the dialysis. Both the partially cross-linked DNA solutions and the

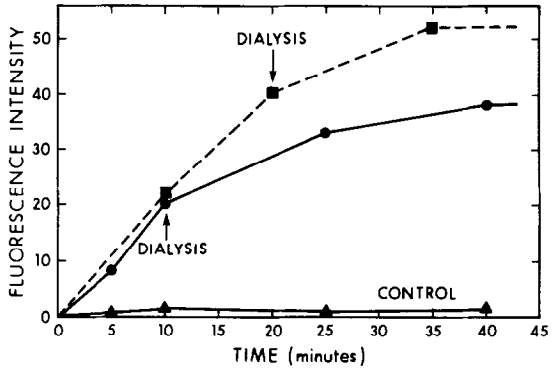

FIG. 6. Stepwise nature of the photoinduced cross-linking of λ-DNA by psoralen at 23°C and pH 7.0. Reaction solutions contained 1.0 A_{260} unit of λ-DNA, 1.85 × 10^{-4} *M* psoralen, 40 m*M* potassium phosphate, and 2% ethanol. Irradiation at 360 nm was continued for periods of 10 (●—●) and 20 min (■—■) as indicated, the reactions were stopped and dialyzed against pH 7.0 phosphate buffer for 48 hr at 3°C, and irradiation was continued. The extents of cross-linking were determined by the CLC assay.

control solution were then irradiated for 30–60 min. Figure 6 shows a marked increase in the extent of cross-linking following irradiation after dialysis.

The fact that cross-links are completed by further irradiation at room temperature confirms that the monoadducts initially formed can still absorb in the 360-nm region and therefore require preferential photoaddition of the furan ring 4′,5′-bond to thymine (compare Fig. 7). This result agrees with that of Dall'Acqua, who found that the proportion of fluorescent adducts (those resulting from addition to the 4′,5′-bond) increases over those formed at the 3,4-bond upon lowering the temperature.

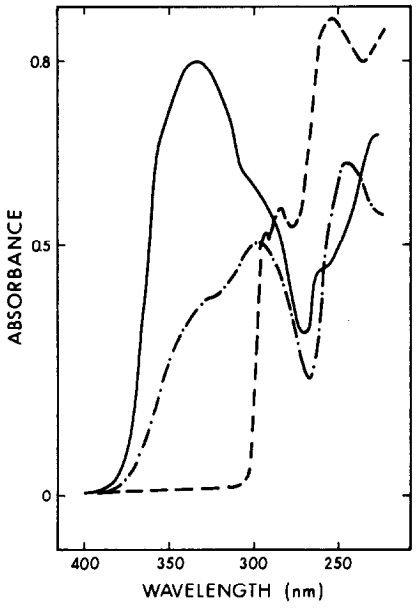

FIG. 7. Absorption spectra of 6×10^{-5} *M* furo[3,2-g]coumarin derivatives in 1% aqueous ethanol. ·—·—·, psoralen; —— 4′,5′-dihydropsoralen; – – – – – 3,4-dihydropsoralen.

Thus temperature effects on photoinduced cross-linking of chromatin by psoralen may also provide useful information on conformational constrains in exposed duplex regions of the macromolecules.

In conclusion, the experiments described herein show how the application of furocoumarins as photoactivated probes, for chromatin structure for example, may be extended by the use of DNA base-specific binding antibiotics, temperature effects to separate monoadduction from cross-linking, and the concept of single photolabeling.

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REFERENCES

1. J. W. DRAKE AND J. MCGUIRE, *J. Virol.* **1**, 260 (1967).
2. L. MUSAJO, G. RODIGHIERO, J. COLUMBO, V. TORLONE, AND F. DALL'ACQUA, *Experientia* **18**, 153 (1962); **21**, 22 (1965).
3. W. L. FOWLKS, D. G. GRIFFITH, AND E. L. OGINSKY, *Nature (London)* **181**, 571 (1958).
4. E. L. OGINSKY, G. S. GREEN, D. G. GRIFFITH, AND W. L. FOWLKS, *J. Bacteriol.* **78**, 821 (1959).
5. M. M. MATHEWS, *J. Bacteriol.* **85**, 322 (1963).
6. L. MUSAJO, F. BORDIN, F. BACCHINETTI, AND R. BEVILACQUA, *Atti. Accad. Naz. Lincei Cl. Sci. Fis. Mat. Natur. Rend.* **43**, 442 (1967).
7. C. COLOMBO AND A. G. LEVIS, *Progr. Biochem. Pharmacol.* **1**, 392 (1965).
8. L. MUSAJO, P. VISENTINI, F. BACCHINETTI, AND M. A. RAZZ, *Experientia* **23**, 335 (1967).
9. T. B. FITZPATRICK, K. A. ARNDT, A. M. ELMOFTY, AND M. A. PATHAK, *Arch. Dermatol.* **93**, 589 (1966).
10. M. A. PATHAK AND D. M. KRAMER, *Biochem. Biophys. Acta* **195**, 197 (1969).
11. L. MASAJO, G. RODIGHIERO, A. BRECCIA, F. DALL'ACQUA, AND G. MALESANI, *Photochem. Photobiol.* **5**, 739 (1966).
12. R. S. COLE, *Biochem. Biophys. Acta* **217**, 30 (1970).
13. L. MUSAJO, F. BORDIN, G. CAPORALE, S. MARCIANI, AND G. RIGATTI, *Photochem. Photobiol.* **6**, 711 (1967).
14. L. MUSAJO, F. BORDIN, AND R. BEVILACQUA, *Photochem. Photobiol.* **6**, 927 (1967).
15. G. P. WIESEHAHN, J. E. HYDE, AND J. E. HEARST, *Biochemistry* **16**, 925 (1977).
16. C. K. J. SHEN AND J. E. HEARST, *Proc. Nat. Acad. Sci. USA* **73**, 2649 (1976).
17. T. R. CECH AND M. L. PARDUE, *Proc. Nat. Acad. Sci. USA* **73**, 2644 (1976).
18. C. S. LEE, R. W. DAVIS, AND N. DAVISON, *J. Mol. Biol.* **48**, 1 (1970).
19. T. CASPERSSON, L. ZECH, AND E. J. MODEST, *Science* **170**, 762 (1970).
20. B. NILSSON, *Heredıtatas* **73**, 259 (1973).
21. A. R. MORGAN AND D. E. PULLEYBLANK, *Biochem. Biophys. Res. Commun.* **61**, 396 (1974).
22. A. H. AKHTAR, A. BEGLEITER, D. JOHNSON, J. W. LOWN, L. MCLAUGHLIN, AND S. K. SIM, *Canad. J. Chem.* **53**, 2891 (1975).
23. A. R. MORGAN AND V. PAETKAU, *Canad. J. Biochem.* **50**, 210 (1972).
24. E. C. HORNING AND D. B. REISNER, *J. Amer. Chem. Soc.* **72**, 1514 (1950).
25. C. G. HATCHARD AND C. A. PARKER, *Proc. Roy. Soc. London* **235**, 518 (1956).
26. V. N. IYER AND W. SZYBALSKI, *Proc. Nat. Acad. Sci. USA* **50**, 355 (1963).
27. D. E. PULLEYBLANK AND A. R. MORGAN, *Biochemistry* **14**, 5205 (1975).
28. J. W. LOWN AND K. C. MAJUMDAR, *Bioorg. Chem.* **6**, 453 (1977).
29. R. B. WOODWARD AND R. HOFFMANN, "The Conservation of Orbital Symmetry," p. 65. Verlag Chemie, 1970.
30. F. DALL'ACQUA, S. MARCIANI, AND G. RODIGHIERO, *Z. Naturforsch.* **246**, 307 (1968).
31. C. ZIMMER, K. E. REINERT, G. LUCK, U. WAHNERT, G. LOBER, AND H. THRUM, *J. Mol. Biol.* **58**, 329 (1971).
32. C. ZIMMER, "Progress in Nucleic Acid Research and Molecular Biology" (J. N. Davidson and W. E. Cohn, Eds.), Vol. 15, pp. 285–312. Academic Press, New York, 1975.
33. G. F. GAUSE, "Antibiotics III, Mechanism of Action of Antimicrobial and Antitumour Agents" (J. W. Corcoran and F. E. Hahn, Eds.), pp. 197–202. Springer-Verlag, New York, 1975.
34. M. A. CHIRIGOS, F. J. RAUSCHER, I. A. KAMEL, G. R. FANNING, AND A. GOLDIN, *Cancer Res.* **23**, 1646 (1963).
35. L. MASAJO AND G. RODIGHIERO, *Photochem. Photobiol.* **11**, 27 (1970).