Sequence Specificity of Psoralen Photobinding to DNA: A Quantitative Approach

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ABSTRACT: The effects of different DNA sequences on the photoreaction of various furocoumarin derivatives was investigated from a quantitative point of view using a number of selfcomplementary oligonucleotides. These contained 5′-TA and 5′-AT residues, having various flanking sequences. The furocoumarins included classical bifunctional derivatives, such as 8-methoxy- and 5-methoxypsoralen, as well as monofunctional compounds, such as angelicin and benzopsoralen. Taking into an account the thermodynamic constant for noncovalent binding of each psoralen to each DNA sequence, the rate constants for the photobinding process to each fragment were evaluated. The extent of photoreaction is greatly affected by the DNA sequence examined. While sequences of the type 5′-$(GTAC)_n$ are quite reactive towards all furocoumarins, 5′-TATA exhibited a reduced rate of photobinding using monofunctional psoralens. In addition terminal 5′-TA groups were the least reactive with 5- and 8-methoxypsoralen, but not with angelicin or benzopsoralen. Also 5′-AT-containing fragments exhibited remarkably variable responses toward monofunctional or bifunctional psoralen derivatives. As a general trend the photoreactivity rate of the former is less sequence-sensitive, the ratio between maximum and minimum being less than 2 for the examined fragments. The same ratio is about 3.4 for 8-methoxypsoralen and 6.2 for 5-methoxypsoralen. This approach, in combination with footprinting studies, appears to be quite useful for a quantitative investigation of the process of covalent binding of psoralens to specific sites in DNA.

Furocoumarins represent an important class of photoactive compounds. In living systems their principal target is DNA, to which they are able to bind covalently upon near UV irradiation (Cimino et al., 1985; Song & Tapley, 1979; Ben Hur & Song, 1984; Rodighiero et al., 1984). The photoreaction process usually involves the 5,6 double bond of thymine and the furan/pyranone double bond of the drug to give monadducts and cross-links of the nucleic acid. Recent investigations on the preference of furocoumarins for binding to specific DNA sequences have shown that these compounds bind preferentially to 5′-TpA residues (Tessman et al., 1985; Gamper et al., 1984; Sinden and Pettijohn, 1984). The effects of DNA sequences were further investigated in the elegant papers of Sage and Moustacchi, 1987, Boyer et al., 1988, and Miolo et al., 1989, in which restriction fragments of natural plasmids were employed and the specificity was investigated by DNA-sequencing methodologies. They found that thymine residues in a GC environment are cold, adjacent thymines are better targets, and alternating $(AT)_n$ sequences are hot spots for photoaddition. For these studies, however, a large excess of the psoralen must be used, and considerable amounts of organic solvent is added to grant homogeneous-phase reactions. Moreover, footprinting methods generate a ranking order of preferential sites which may contain all possible base combinations, but are not suitable for a quantitative description of the extent of noncovalent and covalent binding of the drug in terms of thermodynamic and kinetic parameters.

The DNA sequence specificity for 4,5′,8-trimethylpsoralen cross-linking of DNA has been recently examined in a careful work by Esposito et al., 1988 using ^{32}P-labeled synthetic DNA fragments. A number of possible combinations were considered, having the 5′-TA residue at the center of the sequence. The results suggested short- as well as long-range effects of the composition of the oligonucleotides on the ability of trimethylpsoralen to cross-link. This approach involves the use of saturating concentrations of drug and does not allow a quantitative evaluation of the number of bound psoralen molecules.

To examine the sequence context effects of furocoumarin photobinding under more physiological conditions and with other possible base combinations and to try to get quantitative kinetic data on the overall process, we investigated the covalent photoaddition of various tritium-labeled psoralen derivatives to a number of synthetic oligonucleotides. In this case high DNA/drug ratios can be used while having excellent radiometric responses, and the amount of photobound furocoumarin can be directly determined after removal of unreacted drug and low molecular weight byproducts through simple purification steps. Two types of furocoumarin were examined (Figure 1): two classical bifunctional derivatives, such as 5-MOP[1] and 8-MOP, and two monofunctional (non-cross-linking) compounds, such as ANG or BPS. The synthetic oligonucleotides contain TA or AT residues with different flanking sequences, as shown in Figure 2. In particular they exhibit 5′-TA end groups, T_3A_3, T_2A_2, TATA, or GTAC groups, as well as 5′-AT or A_2T_2 residues. We will try to show that, besides giving further insight into the rules which apply to psoralen photobinding to DNA, our method allows a quantitative approach to evaluate the kinetic parameters for the photoreaction to specific regions of the nucleic acid.

EXPERIMENTAL PROCEDURES

Chemicals. Tritiated 8-MOP, 5-MOP, BPS, and ANG were obtained from AMITY-PG, Amersham (U.K.). They

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[1] Abbreviations: 8-MOP, 8-methoxypsoralen; 5-MOP, 5-methoxypsoralen; ANG, angelicin; BPS, 2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one; TRIS, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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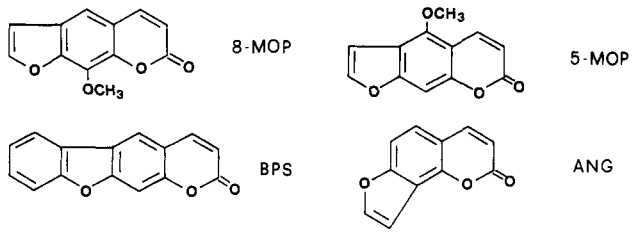


FIGURE 1: Chemical structure of test furocoumarins.

1) T-A-G-C-G-C-T-A

2) C-G-T-T-T-A-A-A-C-G

3) G-C-G-A-T-C-G-C

4) C-G-T-A-T-A-C-G

5) C-G-T-A-C-G-T-A-C-G

6) C-G-A-A-T-T-C-G

7) C-G-T-T-A-A-C-G

FIGURE 2: DNA sequences used in this work (5′ to 3′).

exibited the following specific activities: 8-MOP, 1.97×10^7 dpm μmol^{-1}; 5-MOP, 1.55×10^7 dpm μmol^{-1}; BPS, 2.9×10^7 dpm μmol^{-1}; ANG, 9.02×10^6 dpm μmol^{-1}. They were routinely purified on preparative gel plates (Merck 5717) as reported in previous papers (Palumbo et al., 1986). The structure of the above psoralens is given in Figure 1.

Oligonucleotides (Figure 2). The protected oligonucleotides were prepared in solution according to the hydroxybenzotriazole phosphotriester method (Marugg et al., 1984). Deprotection was performed following the usual protocol (Marugg et al., 1984) and the final crude material purified by anion-exchange chromatography on DEAE-Sephacell eluted with a linear gradient from 0.05 to 2.0 M aqueous triethylammonium bicarbonate, pH = 7.4. Fractions with HPLC purity ≥92–93% were pooled, coevaporated with water and several times with concentrated ammonium hydroxide, and finally lyophylized. HPLC conditions: Spherisorb C_{18} column eluted with a gradient of acetonitrile in 0.05 M KH_2PO_4 buffer (pH = 4.5) at 40 °C.

The following extinction coefficients per residue were used: oligo **1**, 8700 M^{-1} cm^{-1}; **2**, 8700 M^{-1} cm^{-1}; **3**, 8500 M^{-1} cm^{-1}; **4**, 8800 M^{-1} cm^{-1}; **5**, 8300 M^{-1} cm^{-1}; **6**, 8200 M^{-1} cm^{-1}; **7**, 8500 M^{-1} cm^{-1}.

High molecular weight DNA from calf thymus was purchased from Sigma Chemical Co. (St. Louis, MO) and purified as previously reported (Palumbo et al., 1990).

Fluorescence Measurements. BPS exhibits a relatively high fluorescence emission at 440 nm when irradiated at 340 nm. The fluorescence is dramatically quenched upon addition of DNA. This allowed us to characterize the reversible drug–DNA complex by performing titration experiments. Variable amounts of each oligonucleotide were added to a solution at constant drug concentration (of the order of a few μM). Measurements were performed at room temperature on a Perkin-Elmer LS 5 spectrofluorimeter equipped with a Perkin-Elmer 3600 Data Station. The fluorescence response of bound drug was obtained by extrapolating the data at high nucleotide/drug ratios to infinite DNA concentration.

Irradiation Procedures. Small known volumes of concentrated ethanolic solutions of the furocoumarins were added to aqueous solutions (containing 10 mM TRIS, 10 mM NaCl, and 0.5 mM EDTA, pH = 7.0) of each oligonucleotide. The final concentrations of drug were in the 10–20 μM range, whereas the oligonucleotide concentration (per base residue) ranged between 0.5 and 0.8 mM. The amount of ethanol in the final mixture never exceeded 1%. Aliquots of the solutions were transferred into a quartz cuvette and irradiated at constant temperature (20 °C) in a Phototechnology Inst. Model 200-LPS optical apparatus provided with a Osram 150-W lamp. The monochromator wavelength was set at 365 nm. The irradiation intensity was in the range of 50–55 J/s per m^2. After irradiation, each sample was extracted at least six times with chloroform to remove all unreacted material and low molecular weight byproducts. The latter were shown to be soluble in the above solvent when performing irradiation experiments in the absence of DNA.

Radioactivity Measurements. A known volume (0.5 mL) of the solution was added to 10 mL of Scintillator Emulsifer (Packard, Downers Grove, IL) and counted for the evaluation of the radioactivity due to the presence of labeled compounds. A Packard Model A300 CD liquid scintillation spectrometer was used. The efficiency of the apparatus for counting tritium was within 35–40%.

RESULTS

Conformational State of Oligonucleotide Fragments. The melting process of all tested oligonucleotides was investigated spectroscopically in 0.15 M NaCl, at pH 7, as a function of oligomer concentration. The dimerization constant was determined according to the literature (Cantor & Schimmel, 1980). Its value at 20 °C ranged between 7.5×10^5 and 5.5×10^6 M^{-1}, compound **5** giving the most stable duplex and compound **1** the least stable one. The above figures can be easily utilized for evaluating the fractions of oligonucleotide duplex present during the photobinding experiments. They exceeded 0.89 at the lowest concentration of the least stable oligomer. Differences in the extent of strand dissociation can thus be regarded as uninfluential in affecting the drug–oligonucleotide binding process.

Noncovalent Psoralen–Oligonucleotide Binding. Differences in photobinding could arise from different affinities of the psoralen for the reversible interaction with individual DNA sequences. Thus an investigation on the noncovalent DNA binding process is desirable to assess this point. Since the affinity constants for the complexation of furocoumarins to DNA are rather low (Dall'Acqua et al., 1978), the only method which could be generally suitable is equilibrium dialysis with radiolabeled compounds. Trials using cellulose membranes having cutoff of 1000 Da were unsuccessful as the DNA fragments were almost completely absorbed onto the dialysis membrane. Further comparative experiments were attempted where the drugs are dialyzed against high molecular weight DNA in the presence of variable amounts of oligonucleotide, using membranes with 12 000 Da cutoff. Indeed, we were not able to observe an even distribution of the DNA fragments when dialyzed against buffer because of relevant interaction with the membrane. On the other hand, taking advantage of its remarkable fluorometric response and of the relatively high affinity for DNA, it was possible to examine the binding of BPS to each fragment and to natural DNAs having different base composition. The Scatchard plots presented in Figure 3 are very similar in all cases. Thus the reversible interaction between benzopsoralens and DNA does not appear to exhibit appreciable sequence-dependence and each site available for intercalation shows practically the same affinity for the drug. Hence the amount of reversibly bound BPS can be easily calculated from the binding parameters obtained from the Scatchard plots of Figure 3. The extrapolated value for the intrinsic binding constant is about 4200 M^{-1}.

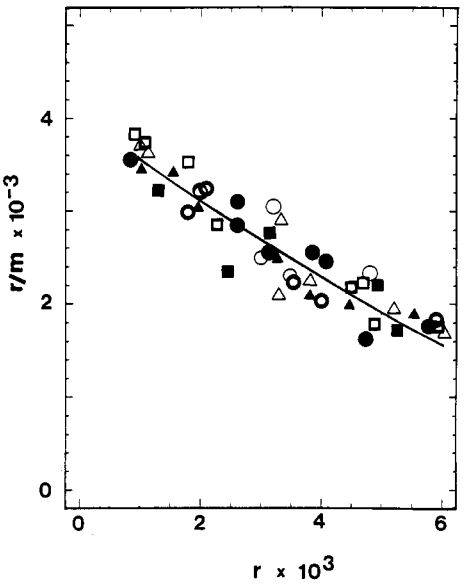


FIGURE 3: Scatchard plot for the binding of BPS to calf thymus DNA (filled circles) and sequences 1–7 (other symbols) in 10 mM TRIS, 10 mM NaCl, 0.5 mM EDTA, pH 7.0. *r* represents the ratio between bound drug and total DNA; *m* represents the free drug concentration at equilibrium.

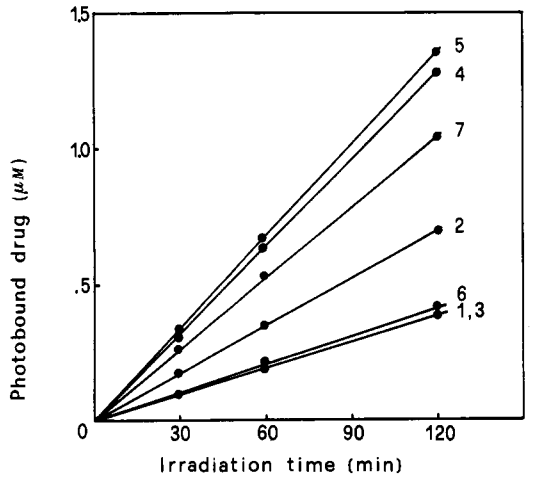


FIGURE 4: Photobinding of 8-MOP to the various oligonucleotide sequences in 10 mM TRIS, 10 mM NaCl, 0.5 mM EDTA, pH 7.0. The concentrations of the psoralen and DNA were 20 mM and 0.49–0.51 mM, respectively.

Photobinding of Psoralen Derivatives to DNA Fragments. The results of photobinding experiments of the examined psoralens to the various oligonucleotides are reported in Figures 4–7 in terms of photoadduct formed as a function of irradiation time. Under our experimental conditions (high nucleotide/drug ratios), a linear trend is observed almost in every case. It is clearly shown that the covalent binding ability of each drug is related to the particular sequence being considered. A striking difference is also observed when comparing the responses of monofunctional vs bifunctional derivatives. In particular for 8-MOP, the overall amount of drug photobound to each fragment (at constant drug and DNA concentration) is in the following order (fragment number is used): **5, 4 > 7 > 2 > 6, 1, 3** (Figure 4). A similar trend is observed for the other bifunctional psoralen, 5-MOP, as shown in Figure 5. On the other hand, fragments **2**, **6**, and **1** were most efficient in the covalent photoreaction with ANG, followed by **5**, **7**, **4**, and **3** (Figure 6). A very similar pattern of reactivity was found for BPS, with the exception of fragment **5**, which binds more efficiently to the tetracyclic furocoumarin (Figure 7).

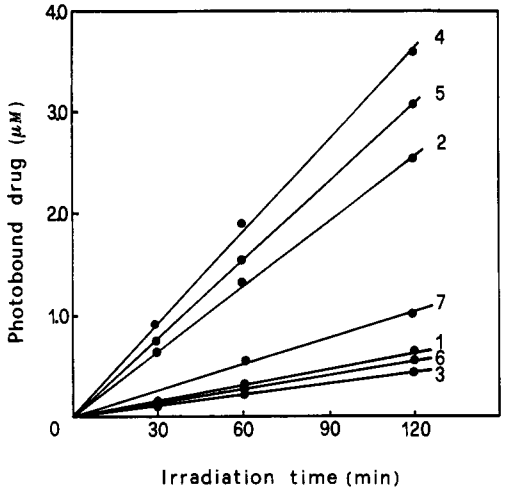


FIGURE 5: Photobinding of 5-MOP to the various oligonucleotide sequences in 10 mM TRIS, 10 mM NaCl, 0.5 mM EDTA, pH 7.0. The concentrations of the psoralen and DNA were 19 μM and 0.51–0.52 mM, respectively.

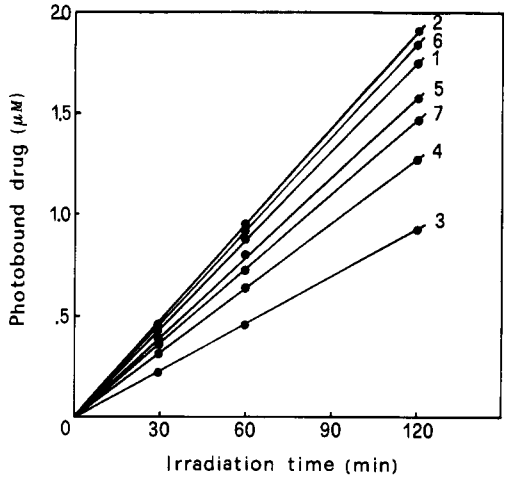


FIGURE 6: Photobinding of ANG to the various oligonucleotide sequences in 10 mM TRIS, 10 mM NaCl, 0.5 mM EDTA, pH 7.0. The concentrations of the psoralen and DNA were 19 μM and 0.58–0.61 mM, respectively.

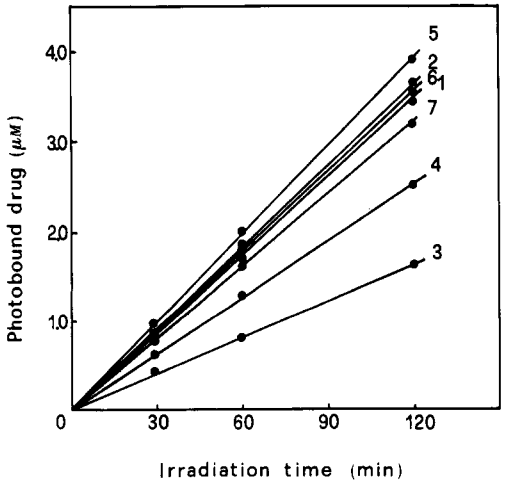
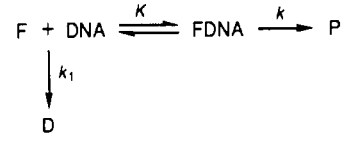


FIGURE 7: Photobinding of PBS to the various oligonucleotide sequences in 10 mM TRIS, 10 mM NaCl, 0.5 mM EDTA, pH 7.0. The concentrations of the psoralen and DNA were 20 μM and 0.51–0.53 mM, respectively.

DISCUSSION

The amount of furocoumarin covalently bound to the nucleic acid fragments is related both to the equilibrium binding

constant and to the rate of photobinding. The process can be simply schematized as follows:



where F represents the furocoumarin, FDNA the reversible furocoumarin–DNA complex, P the photoadducts to DNA, and D the photoreaction products involving the furocoumarin only (dimerization, oxidation, etc.). K is the equilibrium binding constant, k the overall rate constant for the photoreaction to DNA, and k_1 the overall rate constant for the photomodification of the furocoumarin alone.

Considering that $k_1 \ll k$ (Dall'Acqua et al., 1979) and [F] $\ll$ [DNA] under our experimental conditions, the following equation can be derived:

$$d[P]/dT = kKf_{AT}[F_0][DNA_0]/(1 + K[DNA_0]) \quad (1)$$

where $[F_0]$ and $[DNA_0]$ represent the initial drug and nucleic acid concentration. f_{AT} represents the fraction of allowed intercalation sites containing at least one T residue, since negligible covalent binding to GC bases can be reasonably expected under the experimental conditions we used (Sinden et al., 1988).

Integration of eq 1 gives

$$[P] = kKf_{AT}[F_0][DNA_0]t/(1 + K[DNA_0]) \quad (2)$$

which accounts for the linearity of the plots $[P]$ vs t in Figures 4–7. According to eq 2 a k value can be obtained for the binding of each furocoumarin to each duplex sequence, once K values are known.

Whereas for BPS K is experimentally available for all oligomers, as shown in Figure 3, this is not case for the other test drugs. The information available in the literature for the reversible binding of linear and angular furocoumarins points to the following: (a) alternating purine–pyrimidine sequences are largely preferred over nonalternating sequences; (b) the affinity of the drug for alternating (and nonalternating) sequences is practically independent of the nature of the purine or pyrimidine; and (c) the intrinsic binding constant (K_0) for different natural nucleic acids is independent of DNA composition (Dall'Acqua et al., 1978). Point c is a direct consequence of points a and b as the fraction of alternating and consecutive sequences is almost the same ($\simeq$0.5) for the natural DNAs used.

The binding process of 8-MOP, 5-MOP, and ANG to any DNA sequence can thus be described by means of two affinity constants: K_{alt}, which refers to intercalation into binding sites formed by alternating purine–pyrimidine sequences and K_{cons}, for binding sites formed by consecutive pyrimidine or purine residues. Hence the total concentration of reversibly bound drug ($[F_b]$) for a given DNA fragment at a low psoralen/DNA ratio will be related to total DNA concentration (DNA_t) and free drug concentration ([F]) by the following equation:

$$[F_b]/(DNA_t[F]) = K_{alt}f_{alt} + K_{cons}f_{cons} \quad (3)$$

where f_{alt} and f_{cons} represent the fraction of binding sites having alternating and consecutive sequences, respectively. As mentioned before, $K_{alt} \gg K_{cons}$. Hence eq 3 becomes

$$[F_b]/(DNA_t[F]) \simeq K_{alt}f_{alt} \simeq 2f_{alt}K_0 \quad (4)$$

In fact for the natural DNAs investigated (*Micrococcus lysodeikticus*, *Clostridium perfringens*, and calf thymus) for which $f_{alt} \simeq 0.5$, the (previously defined) intrinsic binding constant K_0 is equal to $0.5K_{alt}$. According to these data, $2f_{alt}K_0$ can be used as a reasonable value for K in the reversible binding of 8-MOP, 5-MOP, and ANG to the various oligonucleotides.

Table I: Rate Constants (k, $min^{-1} \times 10^3$) for the Photoaddition of Furocoumarin Derivatives to Different DNA sequences[a]

oligomer	8-MOP	5-MOP	ANG	BPS
1	1.6	1.2	5.2	3.9
2	1.7	2.6	4.5	2.8
3	2.7	1.7	5.3	3.2
4	4.0	6.1	3.4	2.5
5	5.1	7.4	5.8	4.9
6	2.7	1.7	5.5	3.9
7	2.2	1.9	3.8	2.9
DNA[b]	4.4	3.4	4.3	3.2

[a] Radiation dose 55 J/s per m^2. [b] High molecular weight deoxyribonucleic acid from calf thymus.

The k value represents the overall rate constant for the photobinding of a psoralen derivative to an AT (or TA) base pair in a defined sequence context. It should be pointed out that, unlike other techniques thus far used, the present method allows a direct and quantitative determination of the rate at which a drug is covalently bound to a given region of a nucleic acid. On the other hand, the oligonucleotide approach is limited by the presence of possible end group effects and by the impossibility of assessing long-range effects on the photobinding process.

The k values obtained for the systems we investigated are reported in Table I, using K_0 values of 750 M^{-1} for 8-MOP, 1700 M^{-1} for 5-MOP, and 550 M^{-1} for ANG (Guiotto et al., 1984). These data enable, in principle, evaluation of the total amount and the distribution of psoralen photobound to any DNA chain containing the examined sequences. The specificity of drug binding to DNA is evident for all furocoumarins from the kinetic data.

Bifunctional Psoralens. Considering 8-MOP first, the most reactive fragment is **5**, which contains a repeating GTAC motif, followed by **4**, having a TATA sequence, and then by the AATT-containing fragment **6**. This is in turn as reactive as **3**, having a single AT step, followed by **7** (T_2A_2) and **2** (T_3A_3). The least reactive oligomer is **1**, exhibiting terminal TA groups. It appears that alternating purine–pyrimidine stretches having 5′-TA groups represent the hottest spots for 8-MOP photobinding. Another interesting feature is related to the high reactivity of TA groups followed by C. Clusters of Ts on the other hand do not exhibit high reactivity in this case.

The index of selectivity (ratio between maximum and minimum rate constant) is about 3.4, which gives an idea of the level of selectivity which can be expected in a DNA chain containing the examined sequences. Interestingly the data of Esposito et al., 1988 on the effect of flanking sequences on 4,5′,8-trimethylpsoralen cross-linking to 5′-TA give an index of selectivity of 3.6, in excellent agreement with our data.

The results obtained for the photobinding of 5-MOP to sequences 1–7 compare well with those found for 8-MOP, the order of reactivity being **5** > **4** > **2** > **7** > **6**–**3** > **1**. Fragments **2** and **7** are now slightly more active than **6** and **3**. Interestingly 5-MOP seems to be more selective than its isomer 8-MOP for different sequences as the ratio between maximum and minimum rate is remarkably increased (6.2 vs 3.4). Thus the position of the methoxy substituent plays a role in modulating the photobinding preference of the drug.

For both bifunctional psoralens, 5′-TA sequences are very reactive, which confirms previous literature reports (Kanne et al., 1982a,b; Sage and Moustacchi, 1987; Boyer et al., 1988;

Esposito et al., 1988). However, with both 8-MOP and 5-MOP the AT-containing sequences **3** and **6** exhibit k values close to or higher than those found for the TA-containing fragments **1**, **2**, and **7**. This points to the importance of flanking sequences in determining photobinding efficiency at 5′-TA or 5′-AT steps. Moreover, the fact that TA end groups do not represent hot spots for drug photobinding can be related to the lower conformational restriction exhibited by terminal residues in the helix structure. Clearly, for properly assessing sequence context effects in a long DNA chain from oligonucleotide data, the photoreacting base(s) should be appropriately located to avoid end-group effects.

Monofunctional Compounds. In comparison to bifunctional derivatives monofunctional psoralens ANG and BPS behave quite differently. For ANG and BPS, the order of reactivity is practically the same: **5** > **6** > **3** ≃ **1** > **7** > **2** > **4**. The difference in absolute rates accounts for the fact that only one photoreactive site (3,4) is operating in BPS, while both are possibly operating in ANG (Kittler et al., 1980; Dall'Acqua et al., 1981). Fragment **5** is once more the most reactive, but the 5′-AT-containing fragments **6** and **3** now exhibit quite remarkable photobinding, while the "hot" sequence **4** is the least reactive of the series and drops to k values lower than those found for bifunctional psoralens. T_nA_n groups exhibit photoreactivities distinctly higher than for 8- and 5-MOP. Finally TA end sequences dramatically improve their binding efficiency. It is worth mentioning that Boyer et al., 1988 found a photoreactivity pattern for ANG in HN3 DNA qualitatively similar to 8-MOP and 5-MOP. The relative amounts of the various adducts are however distinctly modified. In particular AT-rich sequences are more responsive, in keeping with the above presented findings.

Another interesting fact is a substantial decrease in the index of selectivity, which drops to less than 2 both for ANG and BPS. Thus their preference for specific DNA sequences appears to be reduced in comparison to bifunctional furocoumarins. The reason for this behavior is possibly related to different intercalation geometries exhibited by the angular furocoumarins and consequent modifications in the alignment of the photoreactive double bonds of the drug with respect to the DNA base. Indeed it was shown that also 8-MOP and 5-MOP derivatives exhibit slightly different average orientation in the intercalated complex (Palumbo et al., 1985). Now it is clearly shown that the rate of photobinding is modulated by the sequence flanking the photoreactive site. In fact, 5-MOP is found to be more reactive than 8-MOP for GTAC-containing sequences, while it is substantially less reactive for AT-containing fragments. Thus the overall reactivity is the result of an averaging through all possible combinations of bases and cannot be taken as an absolute indication of intrinsic activity. In connection with this, photobinding to particular DNA sequences could be resonsible for the phototoxic effects found in this class of compounds, which might not be simply related to monofunctional and bifunctional lesions in DNA. More and more evidence is produced that some monofunctional compounds give erythema and other undesired side effects, while other bifunctional derivatives do not. As a matter of fact the pattern of skin toxicity is quite variable among the examined psoralens and might well reflect changes in the preference for lesions to specific sites of DNA.

The data presented in this paper give quantitative evidence for different kinetics of psoralen photobinding to DNA as a function of the sequence context in which the photoactive base is located. Some general rules apply, as discussed before, which integrate other data presented in the literature using different techniques (Sage and Moustacchi, 1987; Boyer et al., 1988; Esposito et al., 1988). Besides evaluating a larger number of base combinations, a future task is to characterize the type and distribution of mono- and diadducts which are formed in a given sequence context. This will require the synthesis of much larger amounts of oligonucleotide fragments than we have presently available. It will be also of interest to use specific monochromatic wavelengths to modulate the formation of 8-MOP and 5-MOP biadducts with thymidine in a given DNA sequence. Hopefully these efforts will allow us to shed further light into the basic laws governing psoralen bioactivity, including toxicity and DNA damage, at the molecular level.

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REFERENCES

Ben-Hur, E., & Song, P. S. (1984) *Adv. Radiat. Biol. 11*, 131–174.

Boyer, V., Moustacchi, E., & Sage, E. (1988) *Biochemistry 27*, 3011–3018.

Cantor, C. R., & Schimmel, P. R. (1980) in *Biophysical Chemistry*, W. F. Freeman & Co., San Francisco.

Cimino, G. D., Shi, Y., & Hearst, J. E. (1986) *Biochemistry 25*, 3013–3020.

Dall'Acqua, F., Terbojevick, M., Marciani, S., Vedaldi, D., & Recher, M. (1978) *Chem.–Biol. Interact. 21*, 103–115.

Dall'Acqua, F., Marciani Magno, S., Zambon, F., & Rodighiero, G. (1979) *Photochem. Photobiol. 29*, 489–495.

Dall'Acqua, F., Caffieri, S., Vedaldi, D., Guiotto, A., & Rodighiero, P. (1981) *Photochem. Photobiol. 33*, 261–264.

Esposito, F., Brankamp, R. G., & Sinden, R. R. (1988) *J. Biol. Chem. 263*, 11466–1472.

Gamper, H., Piette, J., & Hearst, J. E. (1984) *Photochem. Photobiol. 40*, 29–34.

Guiotto, A., Rodighiero, P., Manzini, P., Pastorini, G., Bordin, F., Baccichetti, F., Carlassare, F., Vedaldi, D., Dall'Acqua, F., Tamaro, M., Recchia, G., & Cristofolini, M. (1984) *J. Med. Chem. 27*, 959–967.

Kanne, D., Straub, K., Rapoport, H., & Hearst, J. E. (1982a) *Biochemistry 21*, 816–871.

Kanne, D., Straub, K., Hearst, J. E., & Rapoport, H. (1982b) *J. Am. Chem. Soc. 104*, 6754–6764.

Kittler, L., Hradecna, Z., & Suhnel, J. (1980) *Biochim. Biophys. Acta 607*, 215–220.

Miolo, G., Dall'Acqua, F., Moustacchi, E., & Sage, E. (1989) *Photochem. Photobiol. 50*, 75–84.

Palumbo, M., Capasso, L., Palu', G., & Marciani Magno, S. (1984) *Nucleic Acid Res. 12*, 8567–8578.

Palumbo, M., Rodighiero, P., Gia, O., Guiotto, A., & Marciani Magno, S. (1986) *Photochem. Photobiol. 44*, 1–4.

Palumbo, M., Baccichetti, F., Antonello, C., Gia, O., Capozzi, A., & Marciani Magno, S. (1990) *Photochem. Photobiol. 52*, 533–540.

Rodighiero, G., Dall'Acqua, F., & Pathak, M. A. (1984) in *Topics in Photomedicine* (Smith, K. C., Ed.) pp 317–397, Plenum, New York.

Sage, E., & Moustacchi, E. (1987) *Biochemistry 26*, 3307–3314.

Sinden, R. R., & Pettijohn, D. E. (1984) *J. Biol. Chem. 259*, 6593–6600.

Song, P. S., & Tapley, K. J. (1979) *Photochem. Photobiol. 29*, 1177–1197.

Tessman, J. W., Isaacs, S. T., & Hearst, J. E. (1985) *Biochemistry 24*, 1669–1676.

Welsh, J., & Cantor, C. R. (1987) *J. Mol. Biol. 198*, 63–71.

Zhen, W., Buchardt, O., Nielsen, H., & Nielsen, P. E. (1986) *Biochemistry 25*, 6598–6603.