Orbons, L. P. M., van der Marel, G. A., van Boom, J. H., & Altona, C. (1986) *Eur. J. Biochem. 160*, 131–139.
Patel, D. J., Kozlowski, S. A., Marky, L. A., Rice, J. A., Broka, C., Dallas, J., Itakura, K., & Breslauer, K. (1982a) *Biochemistry 21*, 437–444.
Patel, D. J., Kozlowski, S. A., Marky, L. A., Rice, J. A., Broka, C., Itakura, K., & Breslauer, K. (1982b) *Biochemistry 21*, 428–436.
Patel, D. J., Kozlowski, S. A., Marky, L. A., Rice, J. A., Broka, C., Itakura, K., & Breslauer, K. (1982c) *Biochemistry 21*, 445–451.
Patel, D. J., Kozlowski, S. A., Nordheim, A., & Rich, A. (1982d) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1413–1417.
Pohl, F. M., & Jovin, T. M. (1972) *J. Mol. Biol. 67*, 375–396.
Riazance, J. H., Baase, W. A., Johnson, W. C., Jr., Hall, K. H., Cruz, P., & Tinoco, I., Jr. (1985) *Nucleic Acids Res. 13*, 4983–4989.
Rich, A., Nordeim, A., & Wang, A. H.-J. (1984) *Annu. Rev. Biochem. 53*, 791–846.
Saenger, W. (1984) *Principles of Nucleic Acid Structure*, p 22, Springer-Verlag, New York.
Sklenar, V., & Bax, A. (1987) *J. Am. Chem. Soc. 109*, 2221–2222.
Wagner, G., & Wüthrich, K. (1979) *J. Magn. Reson. 33*, 675–680.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 743–745.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., van der Marel, G., van Boom, J. H., & Rich, A. (1981) *Science (Washington, D.C.) 211*, 171–176.
Watson, J. D., & Crick, F. H. C. (1953) *Nature (London) 171*, 737–738.
Wemmer, D. E., Chou, S. H., Hare, D. R., & Reid, B. R. (1985) *Nucleic Acids Res. 13*, 3755–3772.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, pp 203–223, Wiley, New York.
Xodo, L. E., Manzini, G., Quadrifoglio, F., van der Marel, G. A., & van Boom, J. A. (1986) *Nucleic Acids Res. 14*, 5389–5397.
Zarling, D. A., Arndt-Jovin, D. J., Robert-Nicond, M., McIntosh, L. P., Thomae, R., & Jovin, T. M. (1984) *J. Mol. Biol. 176*, 369–415.
Zarling, D. A., Calhoun, C. J., Hardin, C. C., & Zarling, A. H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6117–6121.

Phased Psoralen Cross-Links Do Not Bend the DNA Double Helix†

Tali E. Haran and Donald M. Crothers*
Department of Chemistry, Yale University, New Haven, Connecticut 06511
Received December 22, 1987; Revised Manuscript Received April 21, 1988

ABSTRACT: Although the chemical reaction of psoralens with nucleic acids is well understood, the structure of psoralen–DNA cross-linked products is still not clear. Model building studies based on the crystal structure of the psoralen–thymine monoadduct suggest that each cross-link bends the DNA double helix by 46.5° [Pearlman, D. A., Holbrook, S. R., Pirkle, D. H., & Kim, S.-H. (1985) *Science (Washington, D.C.) 227*, 1304–1308]. On the other hand, Sinden and Hagerman [Sinden, R. R., & Hagerman, P. J. (1984) *Biochemistry 23*, 6299–6303] find that, in solution, psoralen cross-linked DNA is not bent. Here we use gel electrophoresis to test the validity of the current models. We have synthesized a series of DNA fragments (21–24 base pairs in length), each containing one unique T-A site for 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) cross-linking. Because of an estimated 28° unwinding of the helix by HMT [Wiesehahn, G., & Hearst, J. E. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2703–2707], one expects that the 22-bp cross-linked fragment will be repeated nearly in phase with the average helical screw when multimerized. In that sequence ligation will maximally amplify any deformation to the double helix. We find that the ligated multimers of cross-linked DNA migrate close to the multimers of non-cross-linked DNA on polyacrylamide gels. Our observations place an upper limit of 10° on DNA bending induced by psoralen cross-linking and indicate unwinding by about 1 bp, as well as stiffening of the double helix. These properties are not unexpected for classical intercalators.

The use of psoralens in medicine started with the ancient Hindus, Turks, and Egyptians more than 3000 years ago and has been practiced ever since. In particular, the psoralens (furocoumarins) have been used in the treatment of vitiligo, a disease in which the skin pigment melanin is lost from certain skin areas, and psoriasis, a disorder showing an increased turnover rate of the epidermal tissue [reviewed by Scott et al. (1976) and Ben Hur and Song (1984)]. The successful use of psoralens in medicine has been linked to their ability to cross-link adjacent pyrimidine bases on two strands of the DNA double helix [reviewed by Scott et al. (1976) and Song and Tapley (1979)]. As cross-linking agents psoralens are popular probes of the structure of DNA, RNA, and chromatin [reviewed by Cimino et al. (1985)]. At present we understand the chemical reactions of psoralens with nucleic acids [reviewed by Hearst (1981) and Cimino et al. (1985)], but the spatial arrangment of psoralen cross-linked DNA is not clear.

Understanding the struture of psoralen–DNA complexes is important for the design and synthesis of new and better psoralen derivatives, with greater therapeutic potential and less severe side effects. They can be used in the design of more sensitive probes of nucleic acid conformation and for eluci-

†This work was supported by National Institutes of Health Grant CA15583 (to D.M.C.) and by a Rothschild postdoctoral fellowship (to T.E.H.).

0006-2960/88/0427-6967$01.50/0

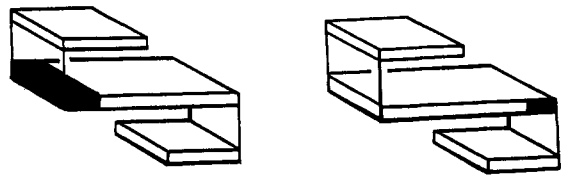

FIGURE 1: Schematic drawing of two cross-linked enantiomers of cis–syn configuration at T-A steps. The large slab is HMT with the five-membered furan ring indicated by the solid area.

dating features recognized by repair enzymes (van Houten et al., 1986).

Psoralen molecules intercalate between the bases of double-stranded DNA or RNA molecules. Upon irradiation by UV light (320–400 nm) two types of monoadduct products can be formed between bifunctional psoralen molecules and pyrimidine bases (mainly thymines). The first monoadduct is a covalent bond between the 4′,5′-double bond of the furan ring and the 5,6-double bond of the pyrimidine base, while the other monoadduct is a covalent bond between the 3,4-double bond of the pyrone ring and the 5,6-double bond of the pyrimidine base. Only the furan-side monoadduct can absorb a second photon (320–400 nm) and form an interstrand cross-link between psoralen and two thymines from adjacent base pairs (Kanne et al., 1982; Tessman et al., 1985). The stereochemistry of both monoadducts as well as the cross-linked product is always cis–syn (Kanne et al., 1982; Figure 1).

Currently, there are two models for the interaction of psoralens with DNA. The first model is based on the crystal structure of a thymine–psoralen monoadduct (Peckler et al., 1982; Pearlman et al., 1985) suggesting that the DNA double helix is bent by 46.5° and underwound by 88° upon cross-linking to psoralen. The second model is a result of a study using the techniques of polyacrylamide gel electrophoresis and differential decay of birefringence (Sinden & Hagerman, 1984). This model proposes that the cross-linking of two thymines by psoralen does not induce large bends in DNA but lengthens the helix by the equivalent of 1 bp. However, these authors were using plasmid DNA, where the cross-linkable sites do not repeat with the helical screw. This causes partial or even total abolishment of the overall DNA bending.

It is the purpose of this work to further address the question of the structure of psoralen–DNA cross-linked complexes. We have studied a series of ligated DNA oligomers of repeating unit length of 21–24 bp. These monomeric molecules were reacted with 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) molecules to form a single unique cross-linked complex at the T-A position in each repeat length. Depending on the degree of unwinding, the T-A sites will be phased constructively in one out of the four sequences, leading to amplification of any deformation in the double helix in the polymer (Koo et al., 1986). We find that the cross-linked and non-cross-linked polymers migrate similarly on polyacrylamide gels and conclude that DNA cross-linked by HMT is bent by less than 10°.

Materials and Methods

Chemicals and Enzymes. 4′-(Hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) was purchased from Calbiochem. It was dissolved in EtOH to make a 4 mM stock solution. [γ-^{32}P]ATP (sp act. >5000 Ci/mmol) was obtained from Amersham. T4 polynucleotide kinase and ligase were obtained from New England Biolabs.

DNA Preparation. The oligonucleotides in Table I were synthesized on an automated DNA synthesizer (Applied Biosystems) by the phosphoramidite method (Dorman et al., 1984). After synthesis, the oligonucleotides were deprotected and purified by electrophoresis on a 20% polyacrylamide–7 M urea gel, followed by EtOH precipitation. All denaturing gels used in this study have a monoacrylamide:bisacrylamide ratio of 19:1 and are run in 0.09 M Tris–borate/2 mM EDTA solution.

Table I: Sequences Used in This Study (5′ to 3′)[a]

name	sequence	length (bp)
TA21	GCGGGCCGGTACCGGCGGCGG	21
TA22	GCGGGCCCGGTACCGGCGGCGG	22
TA23	GCGGGCCCGGTACCCGGCGGCGG	23
AT23	GCGGGCCCGGATCCCGGCGGCGG	23
TA24	GCGGGCCGCGGTACCCGGCGGCGG	24

[a] Only one strand of each duplex is shown. All duplexes were constructed with 4-bp protruding 5′ ends.

5′-End Labeling of DNA Oligonucleotides. A total of 3 μg of each single-stranded DNA oligomer (Table I) was 5′-labeled with 20 μCi of [γ-^{32}P]ATP and 6 units of T4 polynucleotide kinase in a 100-μL solution for 30 min at 37 °C. At the end of that time, 0.5 mM ATP and 6 more units of polynucleotide kinase were added, and the reaction was allowed to proceed for an additional 30 min at 37 °C (Maniatis et al., 1982).

Treatment of Unirradiated Samples. Half of the kinased single-stranded oligomers, 1.5 μg, was passed through an additional 20% polyacrylamide–7 M urea gel, which was run at 65 W until the bromophenol blue (BPB) dye reached the bottom of a 20 cm × 38 cm × 1.6 mm gel. This was followed by EtOH precipitation. The two complementary strands were then hybridized. This additional purification step was carried out in order to make sure that all the oligomers were fully kinased.

Treatment of Samples To Be Irradiated. The remaining 1.5 μg of single-stranded oligomers was hybridized to their complementary strands at the end of the kinasing reaction. The hybridized oligomers were passed through a G15 Sephadex spin column to remove most of the free ATP and irradiated at 4 °C (see below). The cross-linked products were separated from unreacted oligomers, photodamaged DNA, and monoadducts by purifying them on a 20% polyacrylamide–7 M urea gel run at 65 W until the xylene cyanol dye reached the bottom of a 20 cm × 38 cm × 1.6 mm gel. This was followed by EtOH precipitation.

Irradiation Protocol. After the spin column, 1 μg (in 33 μL) of each double-stranded oligomer was mixed with an equal volume of irradiation buffer (50 mM Tris, 0.1 mM EDTA, 10 mM NaCl, 10 mM $MgCl_2$) and 5 μL of HMT/EtOH solution (stock concentration 4 mM) in a small glass vial. The solution was cooled to 4 °C in a jacketed Pyrex beaker filled with circulating water at 4 °C. The solution was irradiated for 20 min with 360-nm monochromated UV light. The Pyrex glass and the water acted as additional filters, such that the contribution of wavelengths below 340 nm was negligible. An additional 5 μL of HMT/EtOH solution was added, and the samples were irradiated for another 20 min.

Ligation and Electrophoretic Analysis. The gel-purified cross-linked products, as well as the purified nonirradiated samples, were multimerized with 2 units of T4 polynucleotide ligase at 16 °C. The overnight reaction was stopped by EtOH precipitation. The concentration of the DNA oligomers in the ligation reaction was approximately 0.1 μM. The ligated products were analyzed on a nondenaturing 8% polyacrylamide gel (monoacrylamide:bisacrylamide ratio 39:1, in 0.09 M Tris–borate/2 mM EDTA) either at room temperature or at 4 °C. The gels were run at 250 V until the BPB dye migrated

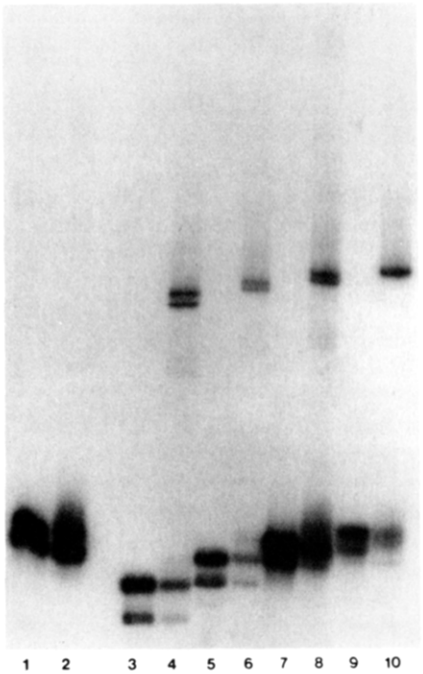

FIGURE 2: Electrophoresis of psoralen cross-linked DNA on a 20% denaturing polyacrylamide gel. Five different sequences are shown pairwise. The first lane of each pair (odd numbers) is the non-cross-linked DNA sample, and the second (even numbers) is the same sample treated with HMT and 360-nm UV light. The cross-linked DNA bands are as follows: X_A (lower band) and X_B (higher band) in TA21–TA23. Lanes 1 and 2, AT23; lanes 3 and 4, TA21; lanes 5 and 6, TA22; lanes 7 and 8, TA23; lanes 9 and 10, TA24. A total of 1 μM of each DNA duplex was treated with 0.2 mM HMT in the dark and subsequently irradiated with 360-nm light at 4 °C for 20 min. An additional aliquot of 0.2 mM of HMT was added, and the sample was irradiated again for 20 min.

30 cm in a 20 cm × 38 cm × 0.8 mm gel.

RESULTS

HMT–DNA Cross-Links. In the cross-linking reaction between HMT and DNA two enantiomeric cross-linked products, X_A and X_B, can be formed from the furan-side monoadduct (Shi & Hearst, 1986; Figure 1). For sequences TA21–TA23 (Table I) these two products are separated on a 20% denaturing polyacrylamide gel (Figure 2). Sequence TA24 however is not resolved into these two enantiomers, even when the cross-linked products run the full length of the gel. The origin of this separation on denaturing polyacrylamide gels, where all secondary structure is lost, is unknown. Therefore, we decided to treat each cross-linked product (X_A and X_B) separately in our assays. For TA23, we have also combined X_A and X_B for ligation. The ligation products co-migrate with the separate cross-linked products (data not shown).

It should be noted that the two enantiomers arise each time from the same 5′...T-A...3′ sequence. The furan side of HMT reacts either with a thymine base in strand 1 or with a thymine base in strand 2 (Figure 1). In addition, Figure 2 shows the attempt to cross-link an AT23 sequence. Earlier observations made by Gamper et al. (1984) and by Sinden and Hagerman (1984) have suggested preferential binding to T-A sequences, over A-T ones. Under identical conditions to those employed for the T-A sequences, the AT23 sequence did not form cross-links with HMT.

Ligated Ladders of HMT–DNA Cross-Links. As mentioned above, each enantiomeric cross-linked product (X_A and X_B) was cut separately from gels, such as the one in Figure 2, and ligated to form multimers from each of the T-A sequences in Table I. Ligation ladders of such multimers of cross-linked DNA are shown in Figure 3, together with ladders of DNA sequences not reacted with HMT. As can be appreciated from Figure 3, the cross-linked multimers are only slightly retarded in the gel relative to their control sequences.

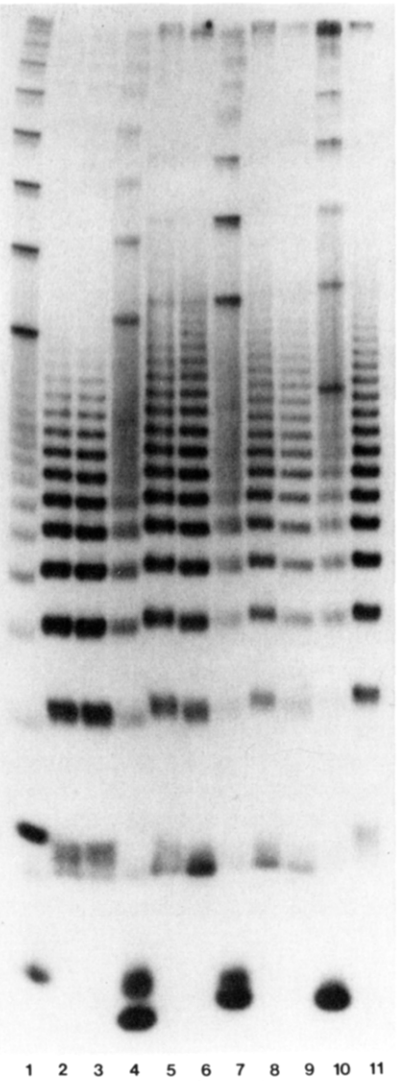

FIGURE 3: Electrophoresis of ligated ladders of cross-linked and non-cross-linked DNA molecules on 8% nondenaturing polyacrylamide gel. Lanes 1, 4, and 7 are multimers of non-cross-linked DNA for TA21, TA22, and TA23, respectively. Lanes 2, 5, and 8 are multimers of cross-linked DNA (X_A, lower band in Figure 2) for TA21–TA23. Lanes 3, 6, and 9 are multimers of cross-linked DNA (X_B, upper band in Figure 2) for TA21–TA23. Lanes 10 and 11 are multimers of non-cross-linked and cross-linked TA24, respectively. Circular products can be seen at the upper part of each non-cross-linked lane.

DNA Stiffness and Circle Formation. Looking at the distribution of linear vs circular products, in cross-linked and non-cross-linked fragments, is another way of assessing the structure of psoralen–DNA complexes. Ligands that interact with the DNA double helix influence the cyclization of DNA fragments. Those ligands that bend the DNA double helix should facilitate the formation of small covalently closed circular products during polymerization. On the other hand, planar aromatic ligands that intercalate between DNA base pairs increase the rigidity of the double helix (Saenger, 1984), leading to decreased formation of small circles.

DNA fragments that are not fully kinased will form nicks when subsequently ligated. We attempted to avoid any such possibility by further purifying the unirradiated sequences after the kinasing reaction. The irradiated sequences, on the other

hand, might have some unkinased ends, as in their isolation step from the gel the fragments are already double helical, and can have either one or two kinased ends. When we look however at Figure 3, we immediately observe that the control sequences have a much higher number of circular products (at the upper part of each lane) than their cross-linked counterparts, indicating greater stiffness in the cross-linked sequence. Since this is true for all values of the helical repeat, the decreased circularization cannot be ascribed to changes in helical phasing.

Discussion

HMT–DNA Cross-Links and Their Ligation Ladders. From the analysis of DNA migration on polyacrylamide gels and from the differential decay of birefringence (DDB), Sinden and Hagerman (1984) have concluded that psoralen-bound DNA is not bent as proposed by Peckler et al. (1982). Instead, it is lengthened by the equivalent of about 1 bp per covalently bound psoralen. For this study, Sinden and Hagerman used a restriction fragment from the plasmid pBR322 in their electrophoretic analysis and a 126-bp restriction fragment from plasmid pXH9 for the DDB experiment. They do not mention the absolute locations of potential cross-linked sites on the pBR322 fragment but note these locations on the 126-bp fragment: 33, 48, 54, 65, 83, 102, and 106. On average, four sites are cross-linked per fragment (Sinden & Hagerman, 1984). As can be immediately realized, these locations are mostly out of phase with the helix screw (10.5 bp per turn). Thus, any attempt to observe psoralen-induced bending in that fragment is hindered by partial cancellation of these bends. For increased sensitivity in the observation of bending, the influence of the cross-linkable sites should be amplified by repetition in phase with respect to the helix screw.

The method employed in our study employs this amplification principle. One out of the four sequences studied has to repeat nearly in phase with the helical screw when the HMT-induced unwinding is taken into account. This sequence when multimerized would show the maximal amount of bending, if it exists. We did not go any further than 24-bp fragments as there were no indications in the literature that psoralens underwind the DNA double helix by more than 100°.

From previously estimated extension and unwinding of the double helix by 1 bp (Wiesehahn & Hearst, 1978; Sinden & Hagerman, 1984), the TA22 fragment should yield multimers that repeat with the helical screw. That this is the case can be evidenced from the circular products on the gel of Figure 3. TA22 is the cross-linked sequence showing the maximum yield of small circular products, thus indicating that it is the one most closely in phase with the helical repeat. Therefore, we can conclude that cross-linked DNA behaves on polyacrylamide gels as if unwound at each cross-link by about 1 bp, compared to the normal DNA helix repeat of about 21 bp.

We observed only slight differencs in retardation on polyacrylamide gels between any of the four cross-linked T-A sequences employed in in this study, compared to their control sequences. Careful examination of the gel in Figure 3 reveals that there is a slight retardation of the cross-linked bands relative to their controls in the nearly in-phase compounds TA21 and TA22. The retardation is smaller in the less well phased species TA24, as expected if the retardation in TA21 is due at least partially to in-phase addition of bends and not exclusively to other effects such as unwinding, lengthening, stiffening, and charge neutralization caused by the intercalated psoralen. The observed ratio R_L of apparent to real base-pair length is about 1.07 for the 147-bp cross-linked TA21 fragment. Assuming that this effect is entirely due to bending and using the calibration equation of Koo and Crothers (1988) yield an upper limit of 10° for the bend induced at each cross-link. However, it is more likely that some of the retardation is due to the above-mentioned effects and that the psoralen-induced bends are significantly less than 10°. If we consider that thermal bending fluctuations of the DNA double helix are about half that large, we can conclude that, to a first approximation, such bends are not significant and the helix can be considered straight.

No apparent change in the migration of the cross-linked vs control sequences have been observed when these same sequences were run on the same gel and under similar conditions at 4 °C (data not shown).

Monoadduct versus Cross-Linked DNA–Psoralen Complexes. The hypothesis of bent psoralen–DNA cross-links was advanced on the basis of a monoadduct complex between thymine and a 8-methoxypsoralen molecule. It can be that monoadduct complexes can bend the double helix, while the final cross-linked products do not. The two cyclobutane rings, formed on two opposite strands, could cancel each others capabilities to bend the double helix or their bends could compensate each other.

It has been shown that the biological effects of a monoadduct and a cross-link are different [reviewed by Ben Hur and Song (1984)]. The mutagenicity, the carcinogenicity, and the therapeutic ability of the two products are different. Moreover, it has been suggested that the therapeutic effects are mediated by cross-links, while the monoadducts are responsible for the carcinogenic risk involved in psoralen therapy (Hanawalt et al., 1982). The fact that cross-links tie the two double-helical strands together while monoadducts do not will obviously contribute to the different biological effects that these two types of complexes show. We cannot however assume a priori that the structure of the two covalent complexes are similar in all other respects. Another reason for the diverse effects of these complexes can be their different structures.

Models for Psoralen Intercalation and Cross-Linking. Prior to covalently cross-linking two thymine bases on opposite strands, psoralen must intercalate between the base pairs of the DNA double helix. Like any other intercalator drug, it extends and unwinds the helix [reviewed by Waring (1981) and by Neidle and Abraham (1984)]. After intercalation the DNA helix is unwound by 28° (Wiesehahn & Hearst, 1978) and is properly positioned for a [2,2] cyclophotoaddition with the psoralen molecule, which takes place in two steps: the first one yielding monoadducts and the second one cross-links. Zhen et al. (1986) have shown that T-A steps in B-DNA are properly positioned for photobinding, if the 28° unwinding at that step is taken into account. The helix extension and unwinding needed for the intercalation step of the psoralen–DNA interaction probably account for a portion of the modest retardation of the cross-linked sequences on the polyacrylamide gel.

To summarize, the stiffness of the cross-linked double helix taken together with the 1-bp unwinding reminds us of intercalation, where bending is not normally observed (Hogan et al., 1979). These properties fully account for the interaction of psoralen with DNA. Therefore, it appears that no new structure needs to be invoked to explain the covalent interaction of psoralens wth DNA.

Acknowledgments

We thank Aino Ruusala and Grace Sun for technical assistance with oligonucleotide synthesis. We also thank Tarmo

Ruusala, Abe Brown, and Marc Gartenberg for valuable disscussions and technical assistance.

REFERENCES

Ben Hur, E., & Song, P.-S. (1984) *Adv. Radiat. Biol. 11*, 131–171.
Cimino, G. D., Gamper, H. B., Isaacs, S. T., & Hearst, J. E. (1985) *Annu. Rev. Biochem. 54*, 1151–1193.
Dorman, M. A., Noble, S. A., McBridge, L. J., & Caruthers, M. H. (1984) *Tetrahedron 40*, 95–102.
Gamper, H., Dietle, J., & Hearst, J. E. (1984) *Photochem. Photobiol. 40*, 29–34.
Hanawalt, P. C., Kaye, J., Smith, C. A., & Zolan, M. (1982) in *Psoriasis* (Farber, E. M., Cox, A. J., Nall, L., & Jacobs, P. H., Eds.) pp 163–171, Grune & Straton, New York.
Hearst, J. E. (1981) *Annu. Rev. Biophys. Bioeng. 10*, 69–86.
Hogan, M., Dattagupta, N., & Crothers, D. M. (1979) *Biochemistry 18*, 280–288.
Kanne, D., Straub, K., Hearst, J. E., & Rapoport, H. (1982) *J. Am. Chem. Soc. 104*, 6754–6764.
Koo, H.-S., & Crothers, D. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1763–1767.
Koo, H.-S., Wu, H.-M., & Crothers, D. M. (1986) *Nature (London) 320*, 501–506.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular Cloning*, pp 122–127, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Neidle, S., & Abraham, Z. (1984) *CRC Crit. Rev. Biochem. 17*, 73–121.
Pearlman, D. A., Holbrook, S. R., Pirkle, D. H., & Kim, S.-H. (1985) *Science (Washington, D.C.) 227*, 1304–1308.
Peckler, S., Graves, B., Kanne, D., Rapoport, H., Hearst, J. E., & Kim, S.-H. (1982) *J. Mol. Biol. 162*, 157–172.
Saenger, W. (1984) *Principles of Nucleic Acid Structure*, Chapter 16, Springer-Verlag, New York.
Scott, B. R., Pathak, M. A., & Mohn, G. R. (1976) *Mutat. Res. 39*, 29–74.
Shi, Y.-B., & Hearst, T. E. (1986) *Biochemistry 25*, 5895–5902.
Sinden, R. R., & Hagerman, P. J. (1984) *Biochemistry 23*, 6299–6303.
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.
van Houten, B., Gamper, H., Holbrook, S. R., Hearst, J. E., & Sancar, A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8077–8081.
Waring, M. J. (1981) *Annu. Rev. Biochem. 50*, 159–192.
Wiesehahn, G., & Hearst, J. E. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2703–2707.
Zhen, W.-P., Buchardt, O., Nielsen, H., & Nielsen, P. E. (1986) *Biochemistry 25*, 6598–6603.

Synthesis and Properties of Novel Psoralen Derivatives

Merrill Goldenberg,[†,§] John Welsh,[‖] Robert Haas,[‡] Darryl C. Rideout,[‡] and Charles R. Cantor*[,‖]

Department of Chemistry, Columbia University, New York, New York 10027, and Department of Genetics and Development, Columbia University College of Physicians and Surgeons, 701 West 168th Street, New York, New York 10032

Received January 11, 1988; Revised Manuscript Received April 19, 1988

ABSTRACT: We have synthesized a set of new trimethylpsoralen derivatives that are characterized by a chain extending from the 4′-position of the furan ring and linked to this ring by an aminomethylene group. The nature of the side chain can be varied widely. In these derivatives, the chains contain either amino or ethylene oxide units for enhanced water solubility and allow the introduction of a thiol or amine group to nucleic acids. These compounds represent the first set of thiolated psoralen derivatives, and their usefulness is demonstrated in several nucleic acid cross-linking experiments. The reagents can be used to create both intraduplex reversible cross-links between the two single-strand partners in a DNA double helix and interduplex reversible cross-links between two DNA double helices.

Psoralens have been used to study nucleic acid structure, damage and repair, and recombination through their involvement in a cross-linking reaction upon irradiation with ultraviolet light (Saffran et al., 1982; Hanson et al., 1976; Wiesehahn et al., 1977). The photochemistry of psoralen has been reviewed extensively (Hearst, 1981; Song & Tapley, 1979; Cantor, 1980). In the dark, psoralens bind to nucleic acids in an intercalative fashion. Upon irradiation with 365-nm light, covalent addition occurs by the formation of cyclobutane adducts between the psoralen and adjacent pyrimidines (Cole, 1970; Kanne et al., 1982). This reaction can lead to the formation of either cross-links between the two strands of a double-stranded molecule or monoadducts to individual strands (Figure 1). Irradiation at 390 nm leads to formation of only monoadducts (Chatterjee & Cantor, 1978). Cross-links can be reversed by irradiation at 254 nm (Rabin & Crothers, 1979).

Psoralen is useful as a structural probe and mutagen partly because its interaction with DNA and RNA is very specific. This allows for modification of nucleic acids in complex nucleoprotein structures, with minimal perturbation caused by reaction with the protein component. Simple psoralen derivatives have been used extensively to study the structure of the ribosome (Wollenzien & Cantor, 1982; Hui & Cantor,

[†] Department of Chemistry, Columbia University.
[§] Present address: Central Research Department, CIBA-GEIGY Corp., Ardsley, NY.
[‖] Department of Genetics and Development, Columbia University College of Physicians and Surgeons.

0006-2960/88/0427-6971$01.50/0