Volume 229, number 1, 73–76 FEB 05595 February 1988

Psoralen photofootprinting of protein-binding sites on DNA

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Received 28 December 1987

Using a BAL31 exonuclease assay to determine the sites of 4,5',8-trimethylpsoralen photocrosslinking in DNA we have shown that 5'-TA sites which are accessible to psoralen DNA interstrand photocrosslinking in naked DNA become inaccessible when protein, in casu, λ-repressor *E. coli* or RNA polymerase are bound at their recognition DNA sequences (O_{R1} operator or *deoI* promoter, respectively). These results show that psoralens can be used as photofootprinting reagents to study specific protein-DNA interactions.

Psoralen; Footprint; RNA polymerase; Promoter; λ-repressor; Photocrosslinking

1. INTRODUCTION

Psoralens have been used extensively to study chromatin structure by electron microscopy (e.g. [1–5]) or by using radiolabeled psoralens (e.g. [6,7]). From these studies it is inferred that the interstrand photocrosslinking by psoralens occurs in 'protein-free' parts of the chromatin exemplified by the internucleosomal linker DNA.

We have recently developed an enzymatic method for determination of psoralen DNA interstrand crosslinks at the nucleotide level [8]. As an extension of this work we now report that proteins such as λ-repressor or RNA polymerase bound to the DNA inhibit psoralen photocrosslinking. Thus, psoralens can be used as

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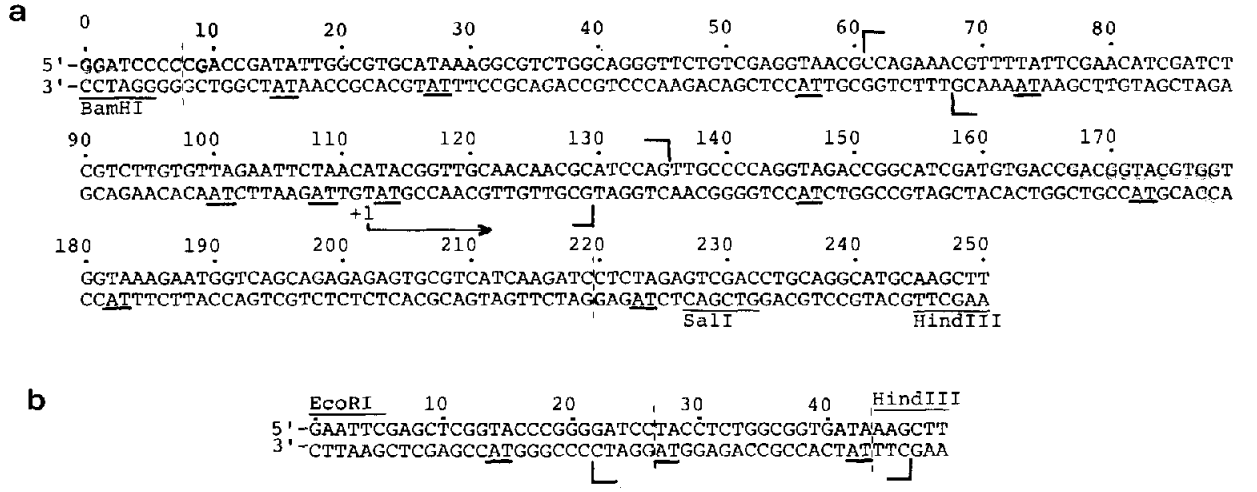

Fig.1. Sequence of the DNA fragments used. (a) *deoI* promoter DNA fragment. Transcription initiation (+1) and direction of transcription (⟶) are indicated. 5'-TA sites are underlined and the DNase I footprint of RNA polymerase binding (⌐ ¬) is also shown. (b) 1-O_{R1} operator DNA fragment with DNase I footprint of λ-repressor (└ ┘) and operator sequence (¦ ¦) shown.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

photofootprinting reagents for studies of specific protein-DNA interactions.

2. MATERIALS AND METHODS

A *Bam*HI-*Bgl*II fragment from pGD11 [9] which contains the *deoI* promoter [10] was cloned into the *Bam*HI site of pUC18 to yield pCJ200.

Two complementary, synthetic 23-mer oligonucleotides constituting the O_{R1} operator DNA [11] having *Hin*dIII/*Bam*HI cohesive ends were cloned into pUC19 via the *Hin*dIII/*Bam*HI sites in the polylinker of this plasmid.

RNA polymerase (purified according to [12]) was a gift from Dr Kaj Frank Jensen, while λ-repressor was purified from an overproducing strain (pEA305 in *E. coli* XA90 [13], a gift from Dr M. Ptashne) according to [14].

Psoralen photoreactions were performed in 50 μl buffer using the desired supercoiled plasmid (0.2 μg) and the samples were irradiated for 25 min at 365 nm (Philips TL 20W/09 fluorescent light tube, 220 $J \cdot m^{-2} \cdot s^{-1}$). The DNA was subsequently purified by phenol extraction and linearized with the first restriction enzyme, treated with BAL31, cleaved with the second restriction enzyme, ^{32}P-end-labeled and analyzed by polyacrylamide gel electrophoresis as in [8]. The buffer for the λ-repressor experiments was 10 mM Tris-HCl, pH 7.0, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM EDTA, 200 mM KCl, 100 μg/ml BSA and 2.5 μg/ml calf thymus DNA. For experiments with RNA polymerase 40 mM Tris-HCl, pH 8.0, 100 mM KCl, 5 mM $MgCl_2$ was used. All samples were incubated at 37°C for 10 min prior to being irradiated.

3. RESULTS AND DISCUSSION

It is well established that 5′-TA sites are much more efficiently crosslinked by psoralens as compared to 5′-AT sites [8,15,16]

The λ-repressor O_{R1} operator DNA sequence contains two 5′-TA psoralen photocrosslinking sites (fig.1, TA_{27}, TA_{42}) and our plasmid construct furthermore has a 5′-TA site 13 base-pairs outside the O_{R1} sequence (fig.1, TA_{14}). The TA_{14} and TA_{27} sites were used to study the effect of λ-repressor binding to the O_{R1} operator on the psoralen DNA interstrand photocrosslinking of TA sites (fig.2). These results clearly show that the TA_{27} site which is efficiently crosslinked in naked DNA becomes completely protected in the presence of λ-repressor.

The absence of detectable crosslinking at TA_{42} is ascribed to the inhibition of enzymatic action by psoralen crosslinks. In this case inhibition of *Hin*dIII cleavage and/or end-labeling by DNA polymerase.

The apparent relative increase in crosslinking of TA_{27} in the presence of repressor is a consequence of the degree of crosslinking (>1 crosslink per fragment), which results in an over-representation of the longer DNA fragments since BAL31 stops at the first crosslink encountered [8].

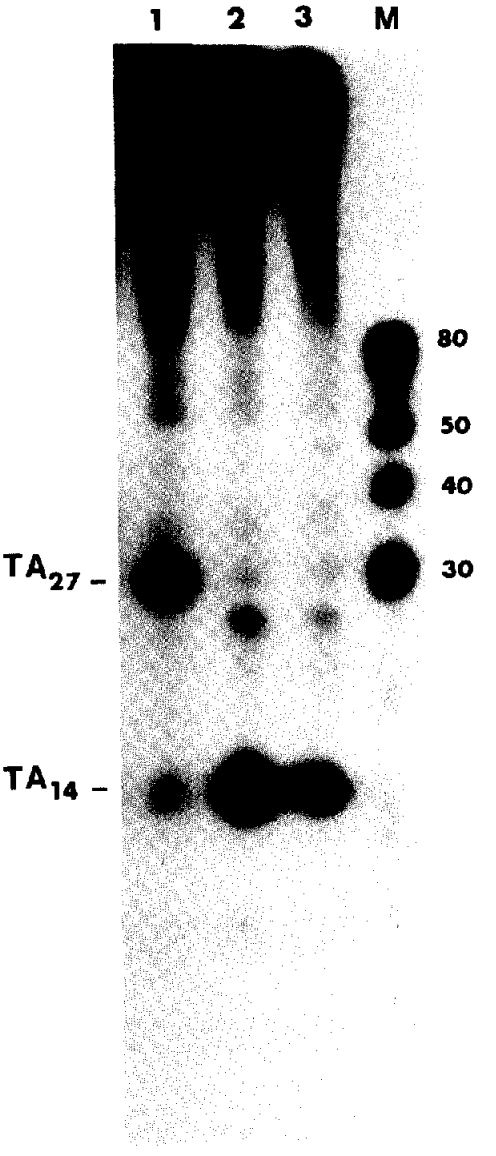

Fig.2. Psoralen photofootprinting of binding of λ-repressor to the O_{R1} operator. The psoralen-treated plasmid was linearized with *Hin*dIII, treated with BAL31, cleaved with *Eco*RI and end-labeled. Lanes: 1, no repressor; 2,3, psoralen-treated in the presence of λ-repressor (0.7 and 2 μg, respectively); M, DNA size marker. DNA analysis was performed on a 20% polyacrylamide gel followed by autoradiography.

The open complex between *E. coli* RNA polymerase and a strong promoter, *deo1*, was used to study the effect of specific protein-DNA interactions on the interstrand photocrosslinking of DNA by 4,5′,8-trimethylpsoralen in more detail, since more psoralen crosslinking sites can be analyzed in this system. The *Bam*HI-*Sal*I DNA fragment used in this study (fig.1) contains 11 5′-TA sites which are all crosslinked by the psoralen (fig.3a, lane 6; fig.3b, lane 5). Furthermore, faint bands (< 10% intensities) are observed at positions corresponding to 5′-AT sites. Upon binding of RNA polymerase complete inhibition of psoralen photocrosslinking occurs at sites TA_{100}, TA_{108} and TA_{113} and AT_{82}, while partial inhibition is seen at TA_{73} (figs 1,2).

DNase I footprinting results have shown that RNA polymerase protects to the −50 to +15 sequence of the *deo1* promoter [17]. Accordingly, 5′-TA sites 100, 108 and 113 are not photocrosslinked with 4,5′,8-trimethylpsoralen when RNA polymerase is bound to the promoter, in either the open or closed (not shown) complex. These results show that tight binding of protein to

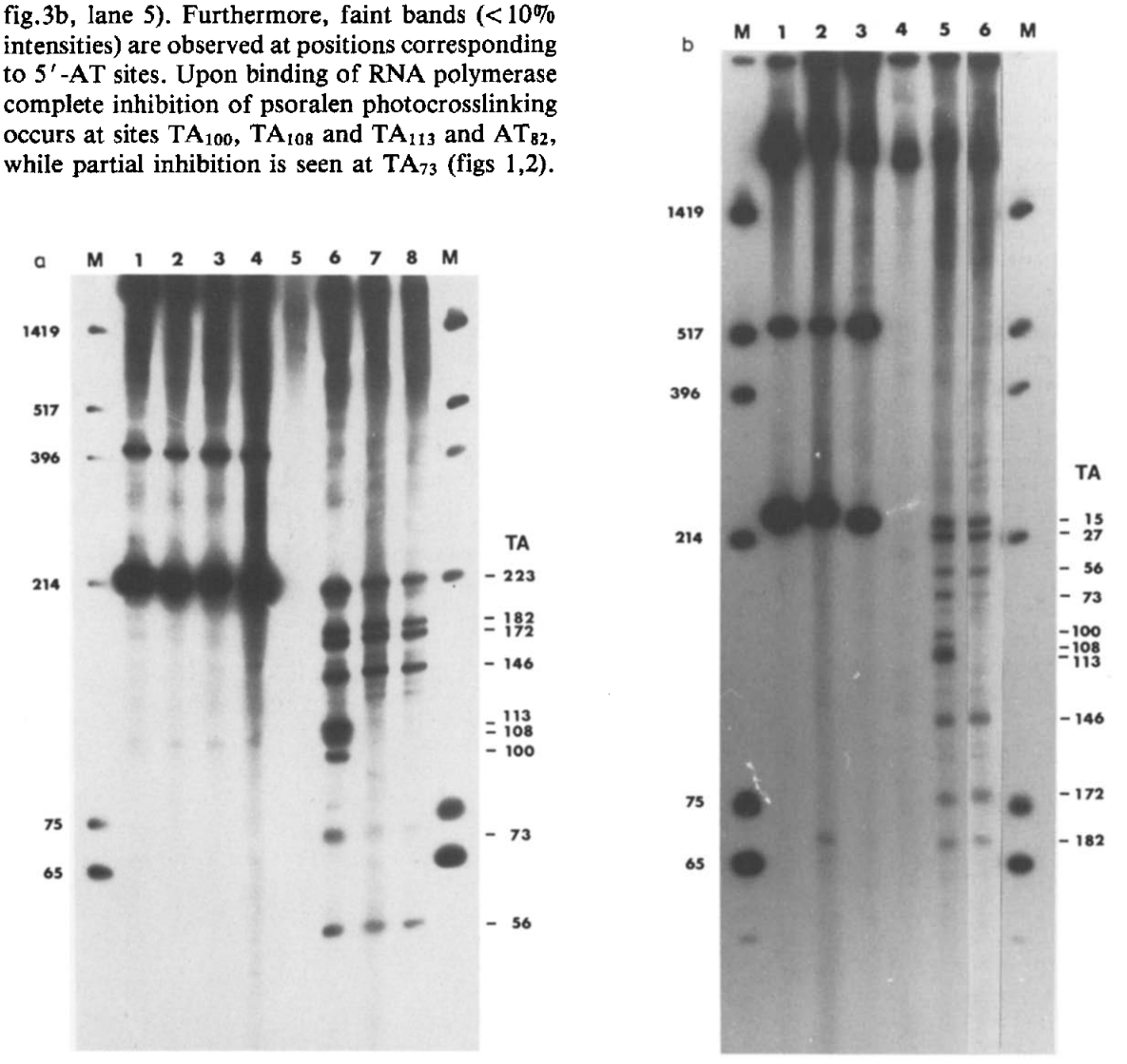

Fig.3. Psoralen photofootprinting of binding of RNA polymerase to the *deo1* promoter. (a) the plasmid (pCJ200) was linearized with *Sal*I, digested with BAL31 and cleaved with *Bam*HI. The labeled DNA fragments were analyzed on a 10% polyacrylamide gel. Lanes: 5, no psoralen, control; 6, psoralen, no polymerase; 7,8, psoralen-treated in the presence of RNA polymerase (3 and 7 μg, respectively); 1–4, as lanes 5–8 but without BAL31 treatment. (b) The plasmid was linearized with *Bam*HI, digested with BAL31 and cleaved with *Hind*III. Lanes: 4, no psoralen, control; 5, psoralen, no polymerase; 6, psoralen-treated in the presence of polymerase; 1–3, as lanes 4–6 but without BAL31. The crosslinking degree was 0.005 per basepair. M, DNA size marker (pUC19 × *Hin*fI).

DNA inhibits psoralen photocrosslinking, presumably due to a rigid conformation of the DNA. This rigidity prevents the helix extension and unwinding which are prerequisites for psoralen intercalation and photobinding.

Interestingly, photocrosslinking of 5′-TA_{73} is only partly inhibited, indicating a less tight association of the enzyme with this part of the promoter. A more loose protein DNA contact around this site is also indicated by DNase I cleavage at positions −34 and −35 [17].

These results clearly show that 4,5′,8-trimethylpsoralen (and presumably other psoralens as well) can be used for photofootprinting studies of protein-DNA interactions, and also firmly establish that psoralens do not induce DNA interstrand crosslinks in DNA which is tightly associated with protein.

Acknowledgements: The financial support of the NOVO Foundation (a Hallas Møller fellowship to P.E.N.), the Weimann Foundation (W.Z.) and the Danish Medical Science Research Council (C.J.) is gratefully acknowledged.

REFERENCES

[1] Hanson, C.V., Shen, C.J. and Hearst, J.E. (1976) Science 193, 62–64.
[2] Wiesehahn, G.P., Hyde, J.E. and Hearst, J.E. (1977) Biochemistry 16, 925–932.
[3] Potter, D.A., Fostel, J.M., Berninger, M., Pardue, M.L. and Cech, T.R. (1980) Proc. Natl. Acad. Sci. USA 77, 4118–4122.
[4] Sogo, J.M., Ness, P.J., Widmer, R.M., Parish, R.W. and Koller, T. (1984) J. Mol. Biol. 178, 897–928.
[5] De Bernardi, W., Koller, T. and Sogo, J.M. (1986) J. Mol. Biol. 191, 469–482.
[6] Cech, T. and Pardue, M.L. (1977) Cell 11, 631–640.
[7] Kondoleon, S.K., Robinson, G.W. and Hallick, L.M. (1983) Virology 129, 261–273.
[8] Zhen, W., Buchardt, O., Nielsen, H. and Nielsen, P.E. (1986) Biochemistry 25, 6598–6603.
[9] Dandanell, G. (1985) Thesis, University of Copenhagen.
[10] Valentin-Hansen, P., Aiba, H. and Schumperli, D. (1982) EMBO J. 1, 317–322.
[11] Humayun, Z., Kleid, D. and Ptashne, M. (1977) Nucleic Acids Res. 4, 1595–1607.
[12] Burgess, R.R. and Jendrisak, J.J. (1975) Biochemistry 14, 4634–4638.
[13] Amann, E., Brosins, J. and Ptashne, M. (1983) Gene 25, 167–178.
[14] Johnson, A.D., Pabo, C.O. and Sauer, R.T. (1980) Methods Enzymol. 65, 839–856.
[15] Gamper, H., Piette, J. and Hearst, J.E. (1984) Photochem. Photobiol. 40, 29–34.
[16] Sage, E. and Moustacchi, E. (1987) Biochemistry 26, 3307–3314.
[17] Jeppesen, C., Buchardt, O., Henriksen, U. and Nielsen, P.E. (1987) submitted.