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Uranyl salts as photochemical agents for cleavage of DNA and probing of protein-DNA contacts

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Single-strand DNA nicks are induced by uranyl nitrate or uranyl acetate in combination with long-wavelength ($\lambda \sim 420$ nm) ultraviolet irradiation. The nicks occur randomly with respect to the DNA sequence. Using the λ-repressor/O_{R1} operator DNA system it is shown that uranyl salts can be used to photofootprint protein contacts with the DNA backbone.

Photofootprinting; Uranyl ion; DNA; Photocleavage; Protein-DNA contact

1. INTRODUCTION

Footprinting techniques have wide applications in molecular biology for the study of sequence-specific binding of proteins to DNA.

DNase I was originally employed as the DNA-cleaving agent for such studies [1,2], and DNase I footprinting is now a standard technique. More recently synthetic DNA-cleaving reagents, such as methidium-EDTA (Fe^{II}) [3] or phenanthroline-Cu^{I} [4], were introduced as footprinting reagents having the advantage over DNase I of giving almost sequence-independent DNA cleavage.

Finally, it has been shown that protein DNA-backbone contacts may be mapped in detail by DNA cleavage with EDTA (Fe^{II}) [5].

Photofootprinting is advantageous over conventional (thermal) footprinting because the DNA-modification reaction (e.g. cleavage) is initiated by an external agent (light) that can be administered within a very short time (by flash irradiation). It is thus much easier to perform low-temperature, time-resolution, or in vivo studies using photofootprinting techniques. Indeed, short-wavelength ultraviolet irradiation alone has been used for in vivo photofootprinting studies [6,7] but interpretation of the results in terms of protein-DNA contacts is not straightforward.

We have recently shown that psoralens [8] or azidoacridines [9] can be employed as photofootprinting reagents, since these DNA intercalators do not photoreact with DNA to which protein is bound.

It is well known that uranyl ions in their excited state are strong oxidizing agents and here we report that light-induced cleavage of DNA is mediated by uranyl salts. This photocleavage is virtually DNA-sequence-independent. Furthermore, we show that such uranyl salts report protein/DNA-backbone contacts, in casu λ-repressor bound to O_{R1} operator.

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2. MATERIALS AND METHODS

Uranyl salts [$UO_2(CH_3COO)_2$ and $UO_2(NO_3)_2$] were of commercial grade and were made up in stock solutions: 100 mM $UO_2(NO_3)_2$ or 50 mM $UO_2(CH_3COO)_2$ in 50 mM HCl.

Two complementary, synthetic 23-mer oligonucleotides constituting the O_{R1} operator DNA [10] having *Hin*dIII/*Bam*HI cohesive ends were cloned into pUC19 via the *Hin*dIII/*Bam*HI

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sites in the polylinker of this plasmid. The plasmid was isolated, purified and 3′- or 5′-end-labeled with ^{32}P at the *Eco*RI site using standard techniques. λ-repressor was isolated [8] from an overproducing strain using published procedures [11].

Plasmid photorelaxation experiments were performed in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA and the DNA was analyzed by gel electrophoresis in 1% agarose (45 mM Tris-borate, 0.5 mM EDTA, pH 8.3), and visualized by ethidium staining.

λ-repressor photofootprinting experiments were performed in 100 μl of 40 mM Tris-HCl, pH 7, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM EDTA, 200 mM KCl containing 0.25 μg calf thymus DNA, 0.7 μg λ-repressor and 0.1–0.2 pmol ^{32}P-labeled DNA fragment. The samples were analyzed by gel electrophoresis (8% polyacrylamide, 50% urea sequencing gels) and the DNA visualized by autoradiography.

Irradiations were performed at room temperature using a Philips TL 40 W/03 fluorescent light tube ($\lambda \sim 420$ nm, 30 nm bandwidth, 20 $J \cdot m^{-2} \cdot s^{-1}$). Samples were irradiated in Eppendorf tubes from above.

3. RESULTS

In the presence of uranyl salts nicking of supercoiled plasmid DNA is induced by long-wavelength irradiation (fig.1). The amount of nicking is dependent on both the uranyl concentration (lanes 2–6) and the light dose (lanes 9–13). The photonicking is not affected by the singlet oxygen quencher, NaN_3 (1 mM, lane 7) or the free radical scavenger dithiothreitol (1 mM, lane 8).

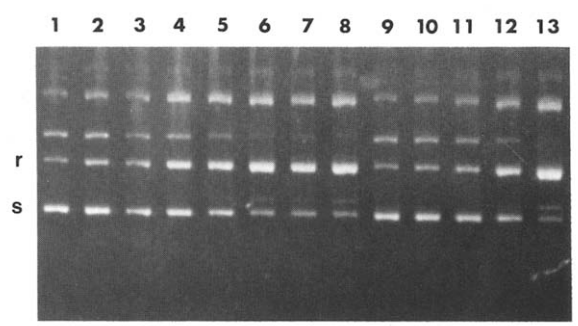

Fig.1. Photonicking of pUC19 DNA by $UO_2(CH_3COO)_2$. A mixture of pUC19 DNA (0.3 μg), in 10 mM Tris-HCl, 1 mM EDTA, pH 7.4 (10 μl), and various concentrations of $UO_2(CH_3COO)_2$ was irradiated and samples were analyzed by gel electrophoresis. Samples for lanes 1–6 contained 0, 0.3, 0.4, 0.5, 0.6 and 0.7 mM $UO_2(CH_3COO)_2$ respectively; irradiation time, 60 min. Samples for lanes 7–13 all contained 0.7 mM $UO_2(CH_3COO)_2$. Those for lanes 9–13 were irradiated for 0, 5, 10, 30 and 60 min, respectively. Samples for lanes 7,8 were irradiated for 60 min and contained 1 mM NaN_3 or 1 mM dithiothreitol, respectively. r, relaxed circular (nicked) pUC19 DNA; s, supercoiled circular pUC19 DNA. The bands above the 'r-band' stem from multimers of the plasmid.

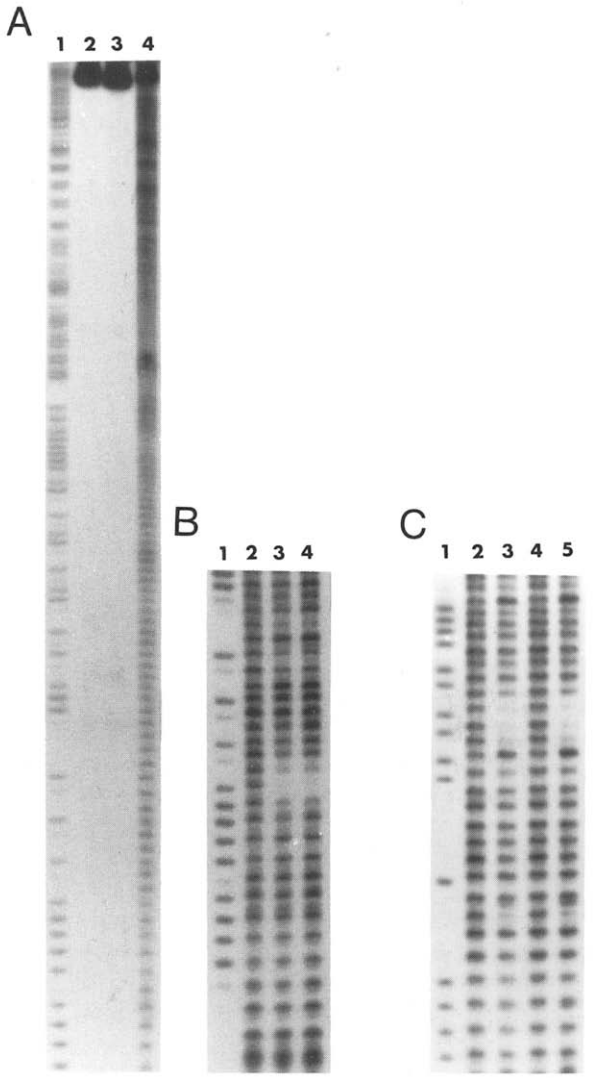

Fig.2. Photocleavage of ^{32}P-end-labeled DNA fragments by $UO_2(CH_3COO)_2$ or $UO_2(NO_3)_2$. The 225 base pair *Eco*RI-*Pvu*II fragment of the O_{R1}-containing pUC19 plasmid was labeled at the (A,B) 3′- or (C) 5′-end at the *Eco*RI site. Samples were analyzed by gel electrophoresis. Lanes: 1 (all panels), A + G formic acid sequence reaction; (A) 2; no uranyl, irradiated; 3, 1 mM $UO_2(CH_3COO)_2$, no irradiation; 4, 1 mM $UO_2(CH_3COO)_2$, irradiated; (B) 2, no repressor; 3,4, 0.7 or 1.4 μg λ-repressor. All samples contained 1 mM $UO_2(NO_3)_2$ and were irradiated; (C) 2, no repressor, 1 mM $UO_2(NO_3)_2$; 3, 0.7 μg λ-repressor, 1 mM $UO_2(NO_3)_2$; 4, no repressor, 1 mM $UO_2(CH_3COO)_2$; 5, 0.7 μg λ-repressor, 1 mM $UO_2(CH_3COO)_2$. The slight difference in gel migration between the fragments of the A + G sequence reaction and those of uranyl cleavage is due to interference by uranyl during electrophoresis. This problem can be avoided by adding 1 mM Na citrate after irradiation prior to ethanol precipitation (not shown). All irradiations were for 30 min.

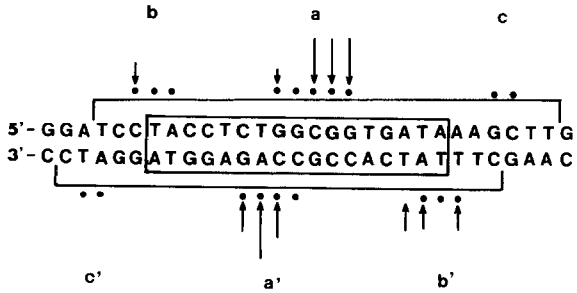

Fig.3. DNA sequence of the O_{R1} operator (boxed in) and sites of protection (arrows) from uranyl photocleavage by λ-repressor. Protection from EDTA (Fe^{II}) cleavage [12] is indicated by dots and DNase I footprint (not shown) by brackets.

The photonicking of DNA by uranyl is practically sequence independent (fig.2a, lane 4) and the resulting DNA fragments migrate as DNA fragments produced by the formic acid sequencing reaction, i.e. as 3′,5′-phosphates.

In the presence of λ-repressor the uranyl photocleavage of O_{R1}-operator DNA is inhibited at very specific sites (fig.2b,c). These sites nearly coincide with those previously detected by EDTA (Fe^{II}) footprinting (fig.3) [12].

4. DISCUSSION

The present results show that uranyl salts induce unspecifically single-strand nicks in DNA upon irradiation with long-wavelength ultraviolet light and that such salts can be used as photofootprinting reagents for analysis of protein-DNA contacts.

The differences in the λ-repressor-O_{R1} footprint produced by uranyl compared to that obtained with EDTA (Fe^{II}) are noteworthy (fig.3). It is believed that EDTA (Fe^{II}) footprints are produced by diffusing hydroxyl radicals [5,12]. A similar mechanism could be operating in the case of the uranyl salts, since the uranyl photooxidation of olefins in H_2O is believed to proceed via hydroxyl radical [13]. However, since uranyl being a cation is expected to bind to DNA, and we find that the radical scavengers dithiothreitol (1 mM, fig.1) or glycerol (2%, not shown) do not influence the photocleavage, it is highly unlikely that diffusing hydroxyl radicals are involved in the photochemical cleavage of DNA by uranyl salts. We therefore suggest that the uranyl salts report accessibility of cations to the DNA double helix. We thus interpret the results of fig.3 in terms of cation displacement at sites a, a′, b and b′ upon binding of λ-repressor to the O_{R1} operator. The actual DNA-cleavage mechanism may, however, still involve hydroxyl radicals produced locally by the DNA associated uranyl ions, but could just as well be a direct oxidation of the deoxyribose units. Both mechanisms could account for the formation of 3′,5′-phosphate ends on the DNA.

From a biological point of view it is interesting that uranyl salts are naturally occurring compounds that have been shown to be phototoxic to bacteria [14]. The DNA-photonicking properties of uranyl salts could contribute to their phototoxicity.

In conclusion, our results show that uranyl salts may turn out to be very useful reagents in molecular biology.

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