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PHOTOCLEAVAGE AND PHOTOFOOTPRINTING OF DNA BY AZIDOACRIDINES

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Introduction

Footprinting of protein binding sites on DNA is a powerful and versatile technique, which can be used both *in vitro* and *in vivo* for studies on protein DNA interactions. Originally DNase I and alkylating agents such as dimethyl sulphate were employed as footprinting reagents [e.g. 1,2]. Recently, however, organic transition-metal complexes with DNA affinity have been introduced as chemical DNA-cleaving and footprinting reagents [3-5]. DNA modification by ultraviolet irradiation has also shown potentials for photofootprinting [6,7].

We are presently designing novel "photonucleases" based on our work with polyfunctional DNA-intercalating 9-aminoacridine derivatives for studies on protein-DNA interactions [8-11].

Results and Discussion

We have previously used azidoacridines in protein-DNA photocrosslinking reagents [10]. In our search for new DNA-photocleaving reagents, which are activated with long wavelength ultraviolet light ($\lambda > 300$ nm), we observed that 9-amino-6-azido-2-methoxyacridines, apart from photoreacting covalently with DNA, also induced single strang nicks in supercoiled plasmid DNA, as well as adducts which upon treatment with hot piperidine give rise to DNA single strand scissions. These scissions occur predominantly at T-residues and to a lesser extent at C and G residues and the products migrate as 5'-phosphates in polyacrylamide gels (Figure 1, lanes 4 & 6). Furthermore, the DNA-photocleavage by reagent 2 is inhibited by protein bound to the DNA as indicated by the protection of promotor DNA by RNA polymerase (Figure 1, lanes 6 & 7). Such protection is less pronounced when reagent 1 is used. However, in this case new cleavage sites are induced at A-7 of the promotor, *e.i.*, the part of the DNA involved in "open complex" formation.

These results clearly indicate that azidoacridines 1 and 2 may be very useful as photofootprinting reagents for studying protein-DNA interactions.

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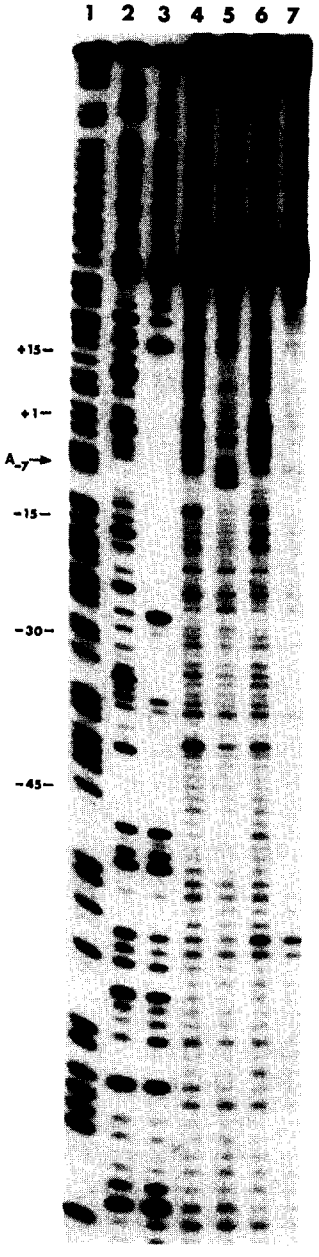

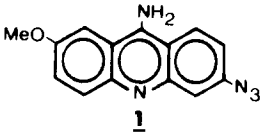

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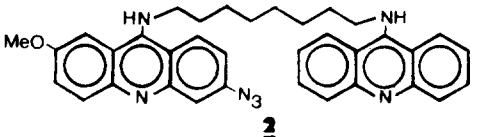

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Figure 1. DNA-photocleavage and photofootprinting of the RNA-polymerase binding site on deo1 promotor DNA (coding strand) [12]. Lane 1: A, G sequence reaction of 3'-end-labeled promotor-DNA. Lane 2-3: partial DNaseI cleavage. Lanes 4-5: photocleavage by **1**. Lanes 6-7: photocleavage by reagent **2**. Lanes 3, 5 & 7: in the presence of *E. coli* RNA polymerase. Transcription initiation is indicated as +1.