Photochemical Cleavage of DNA by 2,7-Diazapyrenium Cations

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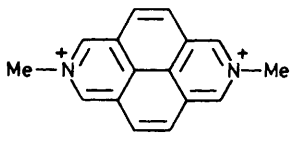
The 2,7-diazapyrenium dication (**1**) and the dimeric tetracation (**2**) bind to double strand supercoiled DNA pBR322 and effect efficient photocleavage under irradiation with visible light, (**2**) being more active than (**1**).

2,7-Diazapyrenium dications such as $MDAP^{2+}$ (**1**) present features resulting from the combination of those of pyrene, of methylviologen, and of nucleic acid intercalators, which define three classes of compounds actively studied for their physical, chemical, and biological properties. These cations strongly bind molecular polyanions and photo-oxidize electron donors under irradiation with visible light.[1] Thence they could (i) interact with DNA, possibly with intercalation, and (ii) effect photocleavage of the DNA strands in visible light by photo-oxidation at the site of binding.

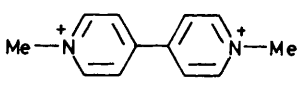
Recent studies of artificial reagents effecting DNA cleavage have made use of metal complexes acting *via* an oxygen dependent redox process[2—4] or by photoactivation.[5—7] Organic intercalating agents like acridine dyes are also known to photodamage DNA.[8]

We now report on the photocleavage of DNA by visible light irradiation in the presence of the dication $MDAP^{2+}$ (**1**) or of the dimer species bis-DAP^{4+} (**2**); for comparison, results obtained with methylviologen MV^{2+} (**3**) itself have also been included.‡

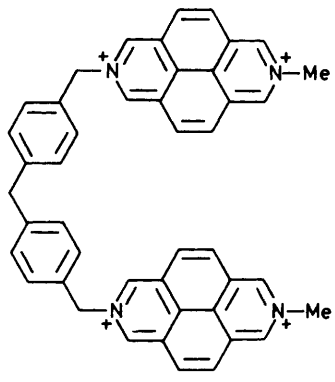
The experiments were performed by illumination for 1 hour with visible light (>395 nm cut-off filter; 250 W slide projector) of a solution containing the supercoiled circular double strand DNA plasmid pBR322 (0.2 μg/μl, *i.e.* about 1.0×10^{-8} M) as well as (**1**) or (**2**) (chloride salts) in Tris-buffer at pH 7.6 and 3 ± 1 °C. DNA cleavage was analysed by gel electrophoresis, monitoring the conversion of supercoiled cDNA into nicked DNA and into linear DNA. The starting supercoiled DNA was first purified so as to contain only a



(**1**) $MDAP^{2+}$



(**3**) MV^{2+}



(**2**) bis-DAP^{4+}

† UA 422 and IBMC (F. X. W.) of the C.N.R.S.

‡ *N,N'*-Dimethyl-2,7-diazapyrenium dication (**1**) was obtained as described in the literature;[9] the preparation of (**2**) will be reported elsewhere together with anion exchange procedures.[1]

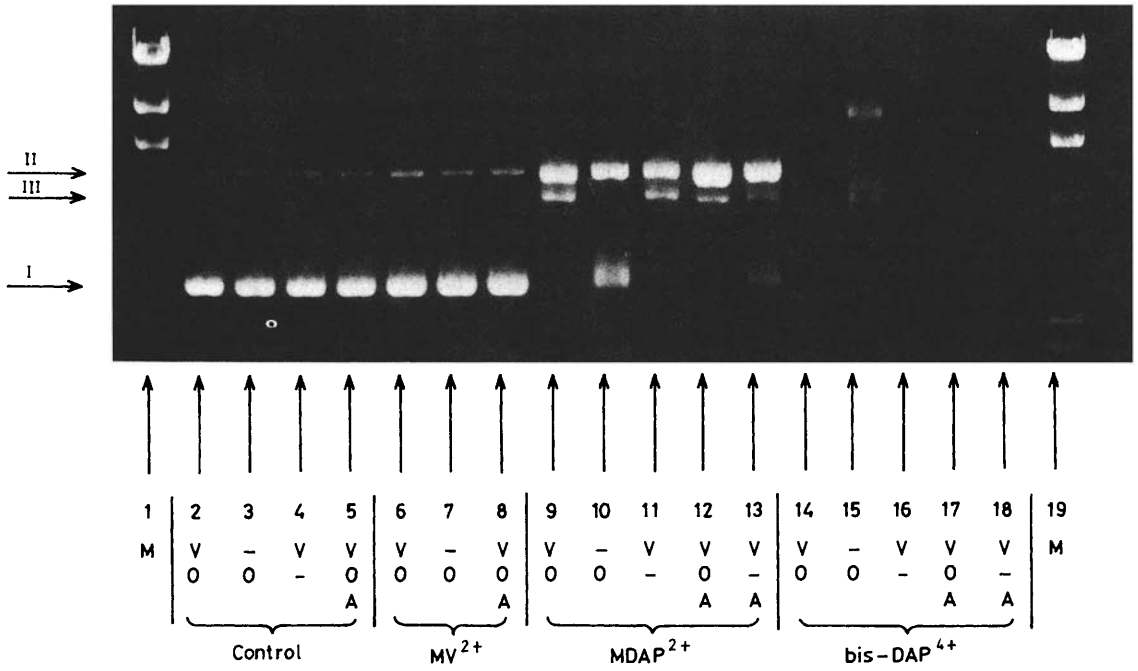


Figure 1. Gel electrophoresis showing results of photochemical cleavage of double strand supercoiled circular cDNA pBR322 (form I) into nicked cDNA (form II) and into linear DNA (form III) by visible light irradiation in the presence of methylviologen MV^{2+} (**3**) (lanes 6—8), $MDAP^{2+}$ (**1**) (lanes 9—13), or bis-DAP^{4+} (**2**) (lanes 14—18). Control experiments in the absence of any agent (lanes 2—5); molecular weight marker from phage λ (lanes 1 and 19) 23106, 9636, 6636, 4433 from top down; V: visible light (>395 nm; 250 W slide projector); O: not degassed, oxygen present; A: EDTA added (1 mM); buffer: Tris (10 mM) at pH 7.6; temperature 3 ± 1 °C. The reactions observed when light was excluded (lanes 10 and 15) may be due to residual stray light during the manipulations; when more complete light exclusion was achieved in other experiments, there was practically no reaction. The marked shifts of the bands observed in lane 15 with respect to those in other lanes, may be attributed to multiple binding of bis-DAP^{4+} reagent. Optical densitometry measurements (corrected for differential staining) gave the following proportions of forms I, II, and III respectively in lane (2): 93, 7, -%; (6): 82, 18, -%; (7): 88, 12, -%; (8): 86, 14, -%; (9): -. 73, 27%; (10) 53, 47, -%; (11): -, 68, 32%; (12): -, 77, 23%; (13): 16, 72, 12%. All irradiations were performed for 1 h; time dependence studies showed that the reactions were already complete after about 20 min.

minimal amount of nicked form. Some of the results are shown in Figure 1. These and other data lead to the following comments.

(a) Experiments with $MDAP^{2+}$ (lanes 9—13, Figure 1): (i) $MDAP^{2+}$ entirely cleaves supercoiled double strand pBR322 DNA under visible light irradiation giving, in the conditions used, mainly nicked circular DNA by cleavage of one strand but also linear DNA by cleavage of both strands when the reaction proceeds further. At lower concentrations of $MDAP^{2+}$ less and less cleavage occurs, transformation into the nicked form being almost complete at 2×10^{-5} M and only slight at 2×10^{-6} M and below. (ii) There is little DNA cleavage in the absence of light. (iii) Removal of oxygen by freeze–thaw degassing somewhat decreases the efficiency of the photocleavage, namely to linear DNA. (iv) Addition of ethylenediaminetetra-acetic acid (EDTA) does not affect the reaction but appears to slow it down when oxygen is removed at the same time. (v) The migration of the pBR322 DNA on the gel is somewhat retarded by the reagent, a possible indication in favour of intercalation.

(b) Experiments with bis-DAP^{4+} (lanes 14—18, Figure 1): (i) in the same conditions as $MDAP^{2+}$, bis-DAP^{4+} shows much more pronounced DNA cleavage, giving small fragments by multiple cuts of both strands. (ii) The reaction is so efficient that suppression of the effect of light becomes difficult; it is nevertheless possible to observe that the migration of pBR322 is markedly retarded, indicating very strong and probably multiple interaction of bis-DAP^{4+} with the DNA. (iii) For the same reason, no effect of O_2 or of EDTA can be seen. At lower concentration the effect of bis-DAP^{4+} decreased markedly, cleavage still being complete at 10^{-5} M and becoming less below.

(c) Experiments with MV^{2+} (lanes 6—8, Figure 1) and control experiments (lanes 2—5, Figure 1) show that in the same conditions, no (or at best very slight) cleavage occurs when MV^{2+} is used and that there is no reaction in the absence of reagent.

These results indicate that both $MDAP^{2+}$ and bis-DAP^{4+} interact with cDNA and effect its photocleavage, the latter very efficiently. Binding to either small molecular polyanions or to DNA is also demonstrated by extinction of the fluorescence of the bound species.[1,10] Although there is no proof that genuine intercalation takes place, this might be the case since these reagents resemble known intercalating compounds like proflavin or ethidium and retard DNA migration on the gel. The binding of pyrene itself to DNA is predominantly intercalative and may present base sequence specificity.[11]

Among compounds possessing two intercalating units,[12—14] a substance containing two phenanthridinium groups linked by a diphenyl ether bridge has recently been shown to act as a double intercalator with DNA.[14] In view of the overall structural analogy, bis-DAP^{4+} may interact similarly and functions in addition as a *doubly photoactive* reagent. Whether the favourable *syn* orientation of the DAP^{2+} units shown in (**2**) is the preferred one in the unbound state is not known at present. However, bis-DAP^{2+} was found to bind molecular anions much more strongly than did $MDAP^{2+}$.[1]

Since DAP^{2+} cations photo-oxidize various substrates (like amines, alcohols, sugars . . .) under visible light irradiation,[1] DNA cleavage might proceed *via* local photo-oxidation at the site of interaction by the excited state of the DAP^{2+} unit. The electron donor may be the ribose unit, since ribose itself has been found to photoreduce $MDAP^{2+}$.[1] The retarding effect of EDTA in the absence of oxygen would indicate that oxidation of this external donor competes with oxidation of the internal donor. The small but not negligible effect of O_2 removal may point to the contribution of an oxygen dependent strand cleavage pathway (as with reduced metal complexes[2—4]); the radical cation species DAP^{+} produced initially by photoreduction[1] could react with O_2, generating superoxide as does MV^{+}.[15] The toxicity of MV^{2+} has been related to DNA damage[16] and to the induction of oxygen dependent free radical processes;[17] the same process may contribute here, especially when both EDTA and oxygen are present. The inefficiency of MV^{2+} for DNA photocleavage may be related to its low photoactivity in visible light[18] and probably also to weaker binding to DNA, compared to $MDAP^{2+}$.§

In conclusion, since DAP^{2+} cations combine binding and photoactivity properties, molecules containing these units are *efficient DNA photocleavage reagents*, which only require *visible light* to effect the reaction. The presence of two such groups as in bis-DAP^{4+}, extends the molecule's potential as a DNA reagent. In particular, two photoactive intercalating groups attached to the ends of a long chain could cut out a fragment of a polynucleotide chain, thus functioning as a *photoexcision reagent* (for a cross-linking reagent see ref. 21), which would possess base sequence specificity if the connecting bridge were a defined oligonucleotide sequence.[22] In this respect, we have recently found that both (**1**) and (**2**) also photocleave single strand circular DNA M13mp19. Attaching a chiral fragment to the photoactive unit might provide a helicity selective reagent since chiral ammonium salts[23] and metal complexes[24] perform such selective interactions with DNA.

Finally, incorporation of photoactive intercalating units into macropolycyclic structures[25] should yield functional receptor molecules of *cyclointercaland* type, endowed with binding selectivity, electroactivity, and photoactivity.

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§ Since acridine dyes have been shown to induce DNA photodamage,[8] a few experiments have been performed on pBR322 cDNA cleavage with *proflavin* PF in the same conditions as $MDAP^{2+}$ (**1**) and bis-DAP^{4+} (**2**). The results obtained indicate that PF is a very efficient DNA photocleavage reagent, comparable to bis-DAP^{4+} and giving (at 2×10^{-4} M) extensive cleavage of DNA beyond the linear form. At lower concentration the efficiency decreased in the sequence $2 \times 10^{-6} > 2 \times 10^{-5} > 2 \times 10^{-7}$ M, which may result from a combination of binding and photoquenching effects.[19] Ethidium bromide on the other hand did not show any photoreactivity in the same conditions. The high efficiency of PF may be due both to its strong intercalative binding to DNA and to its marked absorption in the visible domain ($\lambda_{max.}$ 435 nm, ε 34 000, aqueous solution) which is stronger than that of $MDAP^{2+}$ [1] (see above). The process presumably involves local photo-oxidation of DNA at the site of intercalation with generation of the reduced dye. Acridine dyes are known to undergo photoreduction with substrate oxidation; eventual subsequent reoxidation by oxygen may regenerate the dye.[19,20]

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