Cleavage of DNA Resulting from Exposure to Phenyl Radicals

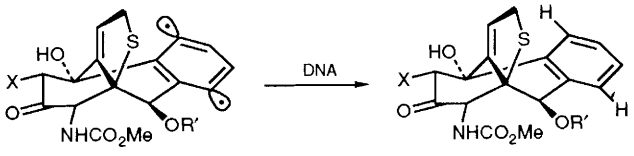
Jonathan Griffiths and John A. Murphy*

Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK

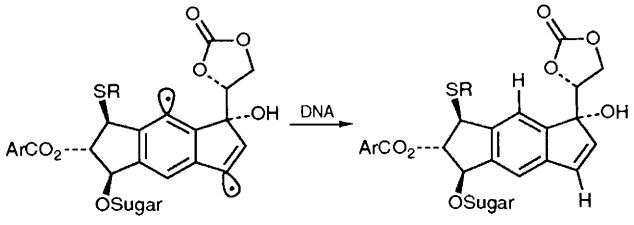
Benzenediazonium tetrafluoroborate has been shown to cleave DNA following single electron transfer from various reagents.

The cleavage of DNA by the antitumour agents neocarzinostatin, calicheamicin and esperamicin has been a focus of research recently. The design of compounds which closely mimic their behaviour has been followed in many laboratories worldwide. These attempts have concentrated on synthesis of ene-diyne structures which could be triggered into Bergman cyclisation to mimic calicheamicin and esperamicin or to undergo the related cyclisation of neocarzinostatin[1] (see Scheme 1). Our experience of free-radical chemistry led us to propose that such complex structures might not be needed,

but rather that any aryl or vinyl radical[2] would be capable of causing the hydrogen atom abstraction reaction from deoxyribose which initiates the scission of DNA.



The reactive diradicals from esperamicin A_1 (X = O-sugar) and calicheamicin γ_1 (X = H) which are responsible for hydrogen atom abstraction from DNA



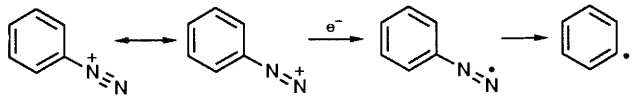
The reactive species from neocarzinostatin which is responsible for hydrogen atom abstraction from DNA

Scheme 1

The second factor which has inspired the present work is that many antitumour agents undergo a reductive conversion into their active form. Thus, it has recently been stated[3] that adriamycin, daunomycin, actinomycin, streptonigrin, saframycin, bleomycin, tallysomicin and mitomycin C all require reductive activation. Their efficacy for antitumour action may be dependent[3] on the fact that 'tumour tissues seem to have a higher reducing potential than normal tissue'. With these ideas in mind, we sought a system where one-electron reduction would produce an aryl radical in the vicinity of DNA leading to DNA cleavage.

The chosen candidate was benzenediazonium tetrafluoroborate. This would be water-soluble, and the positively charged diazonium moiety should be attracted to the negatively charged phosphates on the periphery of a DNA duplex. Delivery of an electron to arenediazonium salts by various reagents is known to be facile,[4] and following loss of nitrogen leads to an aryl radical (Scheme 2).

A previous study[5] of the reaction of diazonium salts with DNA has been conducted. In that study, DNA cleavage was observed, but the mechanism of the reaction was quite different. That reaction was shown to be light-dependent. Aqueous solutions of arenediazonium salts are known to be converted very easily on illumination into aryl cations, which are capable of behaving as alkylating agents. Alkylation of purine bases on DNA is widely used to trigger DNA cleavage.



Scheme 2

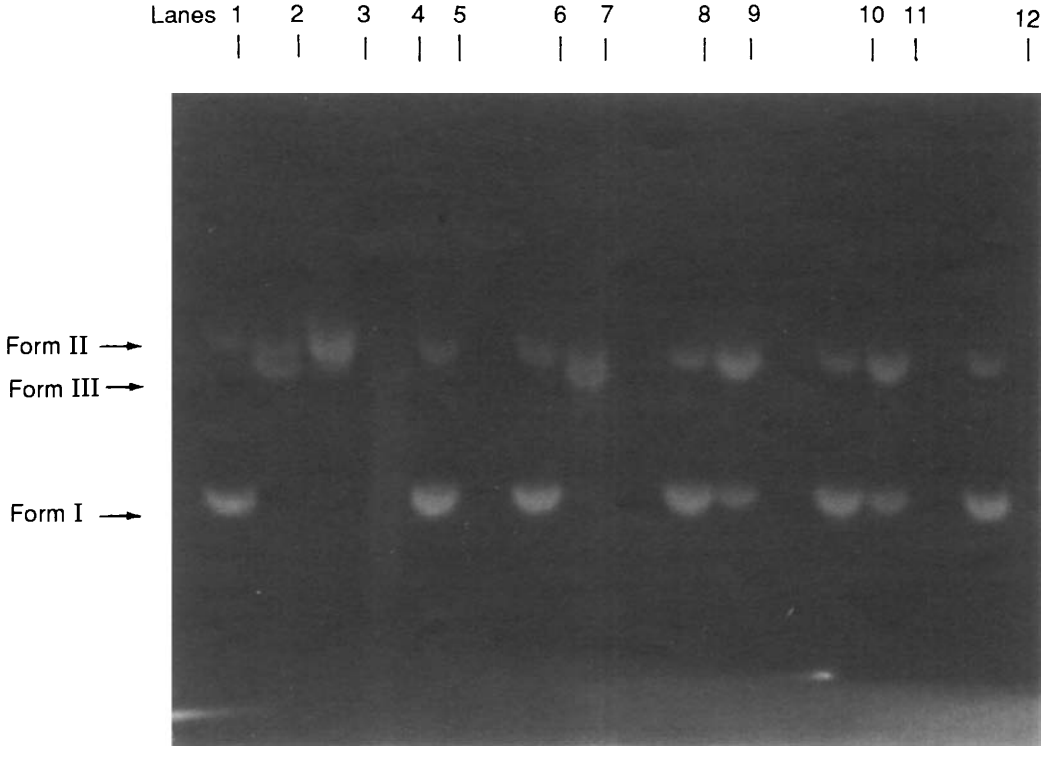


Fig. 1 Lane 1: DNA alone. Lane 2: DNA +0.1 mmol dm^{-3} Fe^{2+} (see ref. 9). Lane 3: DNA + copper(II) + *o*-phenanthroline, 2 min (see ref. 10). Lane 4: DNA + copper(II) + *o*-phenanthroline, 4 min. Lane 5: DNA + 2 mmol dm^{-3} PhN_2^+. Lane 6: DNA + 2 mmol dm^{-3} Cu_2Cl_2. Lane 7: DNA + 2 mmol dm^{-3} Cu_2Cl_2 + 2 mmol dm^{-3} PhN_2^+. Lane 8: DNA + 2 mmol dm^{-3} $CuCl_2$. Lane 9: DNA + 2 mmol dm^{-3} $CuCl_2$ + 2 mmol dm^{-3} PhN_2^+. Lane 10: DNA + 2 mmol dm^{-3} NaI. Lane 11: DNA + 2 mmol dm^{-3} NaI + 2 mmol dm^{-3} PhN_2^+. Lane 12: DNA alone. Loading 445 ng DNA per lane. Reactions were all performed at room temperature in water. Incubations with diazonium salts were performed for one hour using the following final concentrations: 33 nmol dm^{-3} DNA, 830 μmol dm^{-3} PhN_2^+, 830 μmol dm^{-3} one-electron donor. Electrophoresis was performed using 0.7% agarose gel at 50 V for 6 h in the presence of ethidium bromide. Running buffer: TAE (40 mmol dm^{-3} Tris acetate, 1 mmol dm^{-3} ethylenediaminetetraacetic acid), pH 8.2.

We show below that this is not the reaction which we are observing. If a light-induced reaction with DNA were the only reaction, then diazonium salts would not be so potentially useful as therapeutic agents.

In another study, Stock and coworkers[6] investigated the reactions of single nucleosides and nucleotides with diazonium salts. This paper dealt with the formation of triazenes between free NH_2 groups on the bases and the diazonium salts. Radical reactions were seen when these triazenes were heated to high temperatures. Again, we were interested in seeing a room-temperature cleavage of DNA, and not the triazene route. Whereas single nucleosides and nucleotides might easily form triazenes, we felt that it would be more difficult for bases in an ordered stacked duplex to achieve this. It is shown below that the reaction announced in this paper requires an electron-transfer agent.

Accordingly, supercoiled DNA from ΦX174 was exposed to solutions of the diazonium salt in the dark, in the presence of the one-electron donors sodium iodide,[7] copper(I) chloride and copper(II) chloride[8] (it has been suggested that $CuCl_2$, whose reactions with diazonium salts have been extensively studied, contains CuCl and that this allows it easily to act as a one-electron donor). The DNA was also exposed to control solutions of the diazonium salt with none of these additives and also to each of the additives separately with no diazonium salt. The results were probed by electrophoresis (Fig. 1). DNA from ΦX174 has three principal forms: the supercoiled duplex Form I (85% of the commercially available sample is in this form), the nicked duplex Form II which arises when a single strand of the duplex has been cut (present in the initial commercially available samples to about 15%), and the linear Form III which is seen when scission of both strands has occurred in close proximity to each other. As seen in the electrophoretogram, scission to give Form II (or to Forms II and III) is seen only when both the diazonium salt *and* a one-electron donor are present. This shows that the mechanism of cleavage is dependent on electron-transfer.

Arenediazonium salts thus offer a lead into the design of new DNA-cutting molecules of potential use in treatment of viral and neoplastic disorders. It remains to be seen if a diazonium salt can be made to cut DNA with sequence specificity; since this leads to carbon-centred radicals which are non-diffusible (as opposed to hdyroxyl radicals which are more commonly used to cut DNA) it may also be possible to target the reactive radical to a particular hydrogen atom of a particular deoxyribose. Recent studies performed by us[2] indicate that this may have important consequences for the efficiency of DNA cleavage.

Since the completion of these studies, we have seen the publication by Hecht and coworkers of the phenazine di-*N*-oxide system[9] which is also activated by electron-transfer to produce reactive radicals. However, in this case the radicals which cut the DNA are diffusible oxygen-centred radicals.

We thank the SERC for support, and Dr Peter Jones and the Biochemistry Department of Nottingham University for assistance with photography.

Received, 23rd July 1991; Com. 1/03754D

References

1 A. Natrajan, S. M. Hecht, G. A. van der Marel and J. H. van Boom, *J. Am. Chem. Soc.*, 1990, **112**, 3997; J. R. Barr, R. B. van Atta, A. Natrajan, S. M. Hecht, G. A. van der Marel and J. H. van Boom, *J. Am. Chem. Soc.*, 1990, **112**, 4058; P. Magnus, S. Fortt, T. Pitterna and J. P. Snyder, *J. Am. Chem. Soc.*, 1990, **112**, 4986; K. C. Nicolaou, G. Zuccarello, W. Ogawa, E. J. Schweiger and T. Kumazawa, *J. Am. Chem. Soc.*, 1988, **110**. 4866; P. Magnus and P. A. Carter, *J. Am. Chem. Soc.*, 1988, **110**, 1626; P. Magnus, J. Lewis and R. C. Huffman, *J. Am. Chem. Soc.*, 1988, **110**, 6921; J. P. Snyder, *J. Am. Chem. Soc.*, 1989, **111**, 7630; H. Sugiyama, H. Kawabata, T. Fujiwara, Y. Dannoue and I. Saito, *J. Am. Chem. Soc.*, 1990, **112**, 5252.

2 J. Griffiths and J. A. Murphy, *J. Chem. Soc., Chem. Commun.*, 1991, 1442.

3 *Nucleic Acids in Chemistry and Biology*, ed. G. M. Blackburn and M. J. Gait, IRL Press, Oxford, 1990, p. 276.

4 A. J. Fry in *The Chemistry of Diazonium and Diazo Groups*, ed. S. Patai, Wiley, New York, 1978.

5 J. P. Behr, *J. Chem. Soc., Chem. Commun.*, 1989, 101.

6 A. Chin, M.-H. Hung and L. M. Stock, *J. Org. Chem.*, 1981, **46**, 2203; M.-H. Hung and L. M. Stock, *J. Org. Chem.*, 1982, **47**, 448.

7 G. F. Meijs and A. L. J. Beckwith, *J. Am. Chem. Soc.*, 1986, **108**, 5890; A. N. Abeywickrema and A. L. J. Beckwith, *J. Org. Chem.*, 1987, **52**, 2568.

8 M. P. Doyle, B. Siegfried, R. C. Elliott and J. F. Dellaria, Jr., *J. Org. Chem.*, 1977, **42**, 2431.

9 K. Nagai, B. J. Carter, J. Xu and S. M. Hecht, *J. Am. Chem. Soc.*, 1991, **113**, 5099.

10 L. E. Marshal, D. R. Graham, K. A. Reich and D. S. Sigman, *Biochemistry*, 1981, **20**, 244.