Photohydrolysis of DNA by Polyaminobenzenediazonium Salts

Jean Paul Behr

Laboratoire de Chimie Organique Physique, UA 422 du CNRS, Institut Le Bel, 4, rue Blaise Pascal, 67000 Strasbourg, France

Nanomolar concentrations of the *p*-diazonium anilide of L-5-carboxyspermine cleave DNA in daylight *via* a hydrolytic pathway.

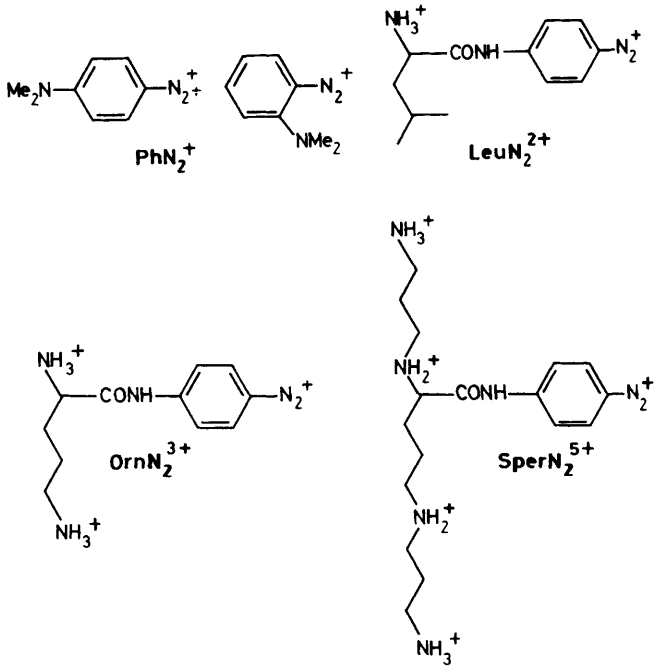
DNA cleavage has been achieved by a variety of small molecules containing a reactive centre and a nucleic acid binding domain, either in a (minor[1] or major groove[2,3]) sequence-specific manner, or with low specificity applicable to footprinting techniques.[4] Single strand cleavage is most often triggered by a redox process[5,6] giving a diffusible free radical, or by (photogenerated[3])-electrophiles[6,7] leading to apurinic sites.

Among photogeneratable electrophile precursors, appropriately substituted arenediazonium salts have been shown to be thermally stable and have been used as protein photolabelling agents;[8] in aqueous media they are converted by light to reactive arene cations with high quantum yields.[9] These react instantaneously with water, but should be able to esterify the phosphodiester backbone of nucleic acids as well, if they are constrained within the vicinity of the polyanion.

Spermine is a tetra-ammonium salt which is of importance *in vivo* in nucleic acid structure and function;[10] in particular, it is known to bind strongly to B-DNA through its cationic groups,[10,11] although the exact structure of the complex is still a matter of debate. In order to make use of it as an anchor group for nucleic acids, it was of general interest to functionalise spermine in such a way as to leave the ammonium residues unmodified. The synthesis of a new amino acid, L-5-carboxyspermine, was therefore undertaken with the aim of using it as nucleic acid-attracting lipid head-group,[12] and as a vehicle for directing molecular groups to the minor groove of B-DNA. This communication reports that the benzenediazonium moiety chemically linked to spermine is able to promote direct photolysis of DNA in a complex formation-dependent manner, down to nanomolar concentrations.

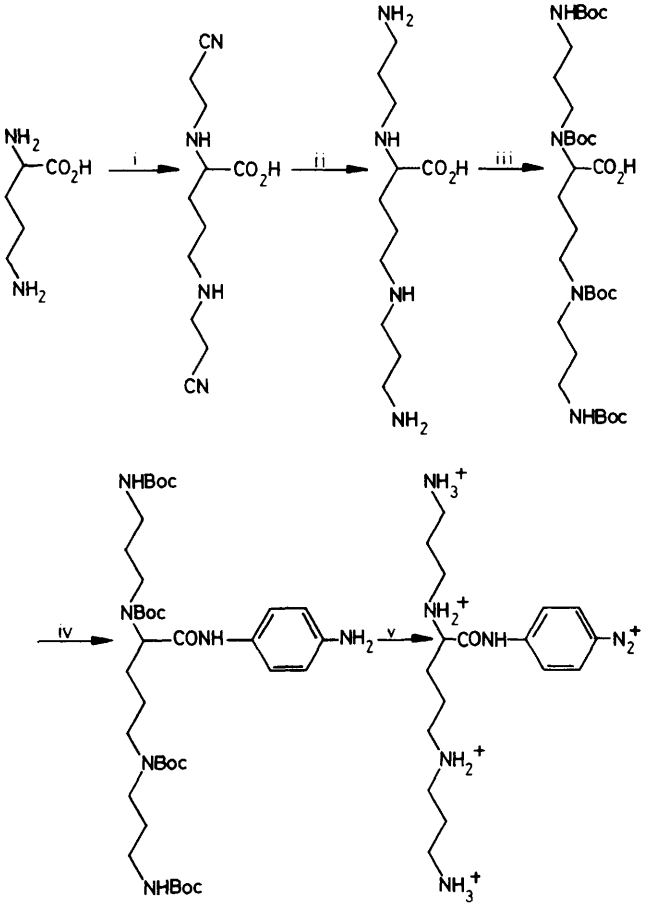
The *p*-diazonium anilides of L-leucine ($LeuN_2^{2+}$), L-ornithine ($OrnN_2^{3+}$), and L-5-carboxyspermine ($SperN_2^{5+}$) were prepared from the corresponding amino acids. The latter compound was obtained (see Scheme 1) through biscyanoethylation of L-ornithine [1 M in dry dimethylformamide (DMF), 2.2 equiv. acrylonitrile, 1 h room temp.] followed by reduction of the dinitrile[13] (H_2, KOH/EtOH, Raney Ni). Amino groups were protected with Boc-ON (Boc = t-butoxycarbonyl) in wet tetrahydrofuran (THF).[14] The t-butoxycarbonylated amino acids were then coupled with excess of 1,4-diaminobenzene (5 equiv. in $CHCl_3$) in the presence of dicyclohexylcarbodiimide (DCC, 1.1 equiv.). Acid extraction and silica gel chromatography yielded the *p*-aminoanilides which were diazotised with isopentyl nitrite (1.2 equiv. in AcOH, 15 min). Finally, deprotection (CF_3CO_2H, 5 min, 0 °C) led to the polyaminobenzenediazonium trifluoroacetates in 55—70% yield from starting amino acids.† The dimethyl-

† ^1H n.m.r. δ (D_2O); $LeuN_2^{2+}$: 1.05 [d, $(CH_3)_2$], 1.85 (m, CH), 1.95 (t, CH_2), 4.35 (t, CHN), 8.20 and 8.65 (AB, Ar); $OrnN_2^{3+}$: 1.85 and 2.10 [m, $(CH_2)_2$], 3.10 (t, CH_2N), 4.30 (t, CHN), 8.20 and 8.65 (AB, Ar); $SperN_2^{5+}$: 1.9 and 2.2 (m, 4 × CH_2 βN), 3.15 (m, 5 × CH_2N), 4.35 (CHN), 8.20 and 8.65 (AB, Ar); ε = 2.25 10^4 dm^3 mol^{-1} cm^{-1} at 323 nm.



aminobenzenediazonium tetrafluoroborates (PhN_2^+) were obtained by diazotisation of the corresponding primary amines with $NaNO_2$ in 30% HBF_4.

DNA single stand cleavage was revealed on agarose gels (Figure 1) by conversion of the supercoiled plasmid pBR322 to the nicked relaxed circular form in the presence of diazonium salts‡ and light.§ Clearly, 10^{-6}—10^{-9} M of the ammonium-bearing diazonium salts cut DNA without need for a subsequent treatment; the reaction is inhibited by cations (lanes 6—8 and data not shown) in the expected order spermine > Mg^{2+} >> Na^+, suggesting common binding sites for all species. The cleavage efficiency is directly related to the complexing ability of the polycation;¶ its concentration dependence is shown in Figure 2†† If no complex is formed between DNA and the arenediazonium species, partition of the highly reactive photointermediate between nucleotides and solvent molecules would rely purely on statistics; here,§ a cleavage probability of $4 \times 10^{-5}/55 \approx 7 \times 10^{-7}$ is estimated. Therefore, the diazonium concentration needed to cleave 4.6 nM of the 4362 base pairs long plasmid is $4.6 \times 10^{-9}/7 \times 10^{-7} \approx$ 6 mM which gives the upper boundary where *any* compound is



Scheme 1. *Reagents:* i, 2 CH_2=$CHCN$, DMF; ii, H_2, Raney nickel, EtOH; iii, Boc-ON, THF; iv, $C_6H_4(NH_2)_2$-*p*, DCC, $CHCl_3$; v, $Me_2CHCH_2CH_2ONO$, AcOH, then CF_3CO_2H.

able to damage DNA provided it is reactive enough. Indeed, this has been found for the simple benzenediazonium salts PhN_2^+ which are not expected to bind to DNA strongly. On the other extreme (Figure 2), the smallest amount of reactant required is obviously fixed by the plasmid concentration itself, the reaction being at its most stoicheiometric. Between these boundaries, the observed cleavage efficiency of the ammonium-bearing diazonium salts parallels their binding to DNA (*i.e.* $LeuN_2^{2+}$ < $OrnN_2^{3+}$ < $SperN_2^{5+}$) in a quantitative manner, the concentration at which cleavage occurs (10^{-4}, 10^{-6}, 10^{-8} M respectively) being roughly equal to the expected affinity in low salt.[11] Thus diazospermine is able to promote daylight-mediated *direct* DNA cleavage at nanomolar concentrations.

The cleavage mechanism is photochemical and probably hydrolytic. Thermal reaction of the diazonium could be ruled out although reaction could not be prevented, even in subdued red light, owing to the high quantum yield and to the sensitivity of the plasmid nicking test. Indeed, a 10^{-5} M solution of $OrnN_2^{3+}$ complexed to calf thymus DNA (10^{-4} M b.p.) is stable with respect to the diazonium (no u.v. change) and the nucleic acid (as seen by gel electrophoresis). Illumination results in a progressive disappearance of the diazonium absorption and concomitant smearing of the nucleic acid band on the gel. On the other hand, the reactive species is not a photoproduct, since a pre-illuminated solution no longer cleaves the plasmid.

‡ The *p*-acylaminobenzenediazonium salts used here are stable in the solid state; in aqueous media they decompose slowly in a base-accelerated fashion ($t_{1/2}$ *ca.* 10 days at pH 4.50).

§ 5 μl of plasmid (40 μM in base pairs) were mixed with 5 μl of reagent in 50 mM tetramethylammonium acetate pH 4.50 buffer, 1 mM NaCl, in subdued red light; photocleavage was complete after 1 min sunlight or slide projector illumination (below 1 μM reagent, diffuse daylight was equally effective). The mixtures were then electrophoresised on a 1% Agarose slabgel which was subsequently stained with ethidium bromide and photographed under u.v. light illumination.

¶ The *meta* diazonium isomer of $LeuN_2^{3+}$ showed no distinct behaviour.

†† The photographs were quantitated by microdensitometry and corrected for a 20% lighter stainability of the supercoiled form. The initial plasmid contained already 15% of the nicked form.

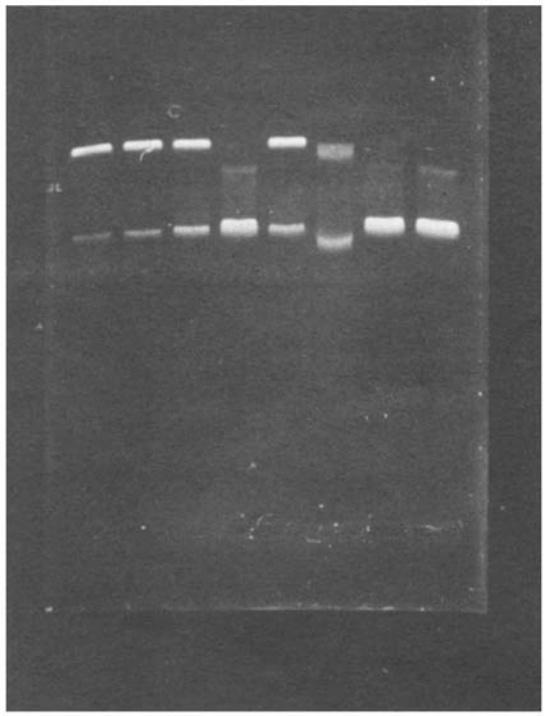


Figure 1. Agarose gel electrophoresis§ of pBR 322 (lane 1, left), in the presence of 0.5 μM $LeuN_2^{2+}$, $OrnN_2^{3+}$, and $SperN_2^{5+}$ (lanes 2—4), 5 nM $SperN_2^{5+}$ (lane 5), and 0.5 μM $SperN_2^{5+}$ with 1 mM spermine (lane 6), 1 mM Mg^{2+} (lane 7), and 10 mM Na^+ (lane 8).

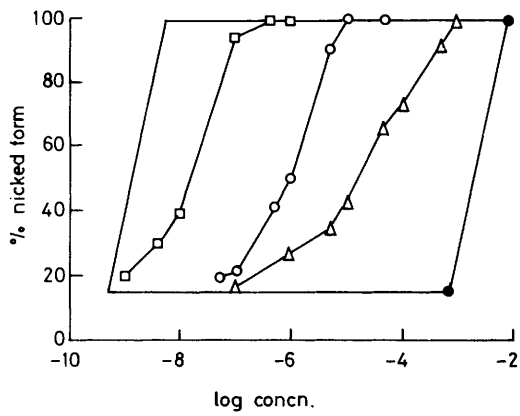


Figure 2. Concentration dependence of the cleavage efficiency.§†† □, $SperN_2^{5+}$; ○, $OrnN_2^{3+}$; △, $LeuN_2^{2+}$; ●, PhN_2^+.

Irradiation of the *p*-acetylaminodiazonium unit in aqueous solution yields the phenol and nitrogen, *via* the carbocation.[9,15] The same conclusion holds for $OrnN_2^{3+}$, as deduced from u.v. absorption and ^1H n.m.r. spectroscopy. In the presence of excess of calf thymus DNA, no shift in the spectroscopic characteristics of the substrate occurs, which strongly disfavours the possible intercalation of the diazonium moiety between the nucleic acid base pairs; only line broadening of the aromatic AB system at δ 8.2 and 8.6 is indicative of restricted motional freedom of the substrate bound to the polymer. Photochemical DNA cleavage broadens the signals even more and shifts the aromatic protons to δ 6.9 and 7.4, values close to that of the phenol (δ 7.0 and 7.4). Finally, hot base treatment (1M LiOH, 80 °C, 1 min) gave identical phenolate spectra (δ 6.7 and 7.2) for the diazonium solutions irradiated with or without DNA. Although cleavage through a minor n.m.r.-silent pathway cannot be ruled out, this experiment is in favour of *hydrolytic* cleavage of an intermediate phosphate triester, as in the case of the RNA structural probe ethylnitrosourea,[16] where the reactive species is thought to be the ethyldiazonium cation.[17]

With the current growing interest in the synthesis of molecules able to hydrolyse[18] DNA or to cleave both strands simultaneously,[19] a functionalised spermine will serve as a very efficient vector to bring appropriate molecular groups close to DNA.

Received, 12th July 1988; Com. 8/02816H

References

1 For a review see P.-B. Dervan, *Science*, 1986, **232**, 464.
2 H. E. Moser and P. B. Dervan, *Science*, 1987, **238**, 645; J. P. Sluka, S. J. Horvath, M. F. Bruist, M. I. Simon, and P. B. Dervan, *ibid.*, 1129.
3 T. Le Doan, L. Perrouault, D. Praseuth, N. Habhoub, J. L. Decout, N. T. Thuong, J. Lhomme, and C. Hélène, *Nucl. Acids Res.*, 1987, **15**, 7749.
4 M. D. Kuwabara and D. Sigman, *Biochemistry*, 1987, **26**, 7234; M. W. Van Dyke, R. P. Hertzberg, and P. B. Dervan, *Proc. Natl. Acad. Sci. USA*, 1982, **79**, 5470.
5 See for instance S. M. Hecht, *Acc. Chem. Res.*, 1986, **19**, 383; J. K. Barton and A. L. Raphael, *J. Am. Chem. Soc.*, 1984, **106**, 2466; J. W. Lown and A. V. Joshua, *J. Chem. Soc., Chem. Commun.*, 1982, 1298; J. P. Hénichart, R. Houssin, J. L. Bernier, and J. P. Catteau, *ibid.*, 1982, 1295; A. J. Blacker, J. Jazwinski, J.-M. Lehn, and F. X. Wilhelm, *ibid.*, 1986, 1035; C. Ohuigin, D. J. McConnell, J. M. Kelly, and W. J. M. van der Putten, *Nucl. Acids Res.*, 1987, **15**, 7411.
6 J. W. Lown, *Acc. Chem. Res.*, 1982, **15**, 381.
7 D. G. Knorre and V. V. Vlassov, *Progr. Nucleic Acids Res. Mol. Biol.*, 1985, **32**, 292; B. F. Baker and P. B. Dervan, *J. Am. Chem. Soc.*, 1985, **107**, 8266.
8 M. P. Goeldner and C. G. Hirth, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 6439; A. M. Tometsko, J. Turuka, and J. Comstock, *Int. J. Pept. Protein Res.*, 1978, **12**, 143.
9 W. Ando, in 'The Chemistry of Functional Groups: Diazonium and Diazo,' S. Patai, Wiley, New York, 1978, p. 341.
10 C. W. Tabor and H. Tabor, *Annu. Rev. Biochem.*, 1984, **53**, 749.
11 W. H. Braunlin, T. J. Strick, and M. T. Record, Jr., *Biopolymers*, 1982, **21**, 1301; J. E. Morgan, J. W. Blankenship, and H. R. Matthews, *Arch. Biochem. Biophys.*, 1986, **246**, 225.
12 J.-P. Behr, *Tetrahedron Lett.*, 1986, **27**, 5861.
13 R. J. Bergeron and J. R. Garlich, *Synthesis*, 1984, 782.
14 M. Itoh, D. Hagiwara, and T. Kamiya, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 718.
15 B. Kieffer, M. P. Goeldner, and C. G. Hirth, *J. Chem. Soc., Chem. Commun.*, 1981, 398.
16 B. Singer and H. Fraenkel-Conrat, *Biochem.*, 1976, **14**, 772.
17 G. P. Margison and P. J. O'Connor, in 'Chemical Carcinogens and DNA,' vol 1, ed. P. L. Grover, CRC Press, 1978, p. 111.
18 L. A. Basile, A. L. Raphael, and J. K. Barton, *J. Am. Chem. Soc.*, 1987, **109**, 7550.
19 P. G. Schultz and P. B. Dervan, *J. Am,. Chem. Soc.*, 1984, **105**, 7748; L. A. Basile and J. K. Barton, *ibid.*, 1987, **109**, 7548.