DNA Cleaving Ability of 9-Diazofluorenes and Diaryl Diazomethanes: Implications for the Mode of Action of the Kinamycin Antibiotics

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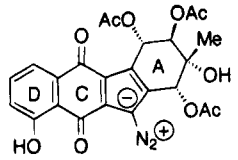
As part of an effort to develop new reagents for DNA cleavage, we recently reported[1] that arenediazonium compounds induce DNA cleavage upon activation with cuprous salts or light. Although highly efficient DNA cleaving activities were recorded,[1] their instability and the need to use extraneous elements, such as metal ions or light, may severely limit the therapeutic potential of these compounds. The isolation of diazobenzo[*b*]fluorenes[2,3] as stable antitumor natural products (e.g., kinamycin C **1** and prekinamycin **2**) aroused our interest because diazo groups are simply the deprotonated forms of the diazonium compounds. We speculated that pro-



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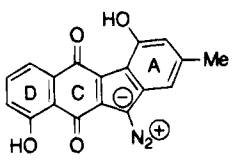

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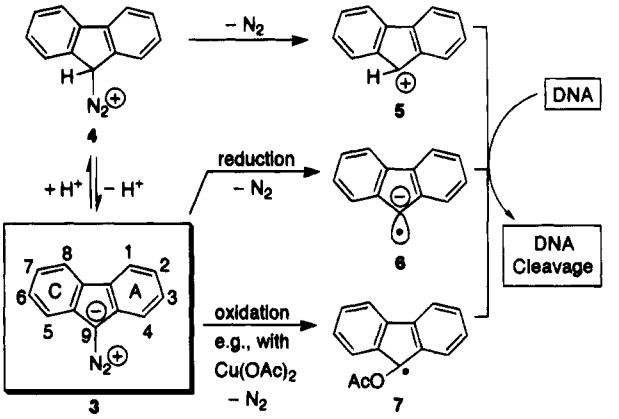
tonation, perhaps under physiologically relevant pH's, may generate unstable diazonium ions[4,5] which, after a spontaneous loss of nitrogen (N_2), should generate carbocations capable of cleaving DNA via alkylation. However, two other mechanisms may also be envisioned: (i) Reduction[4] (e.g., by metal ions) followed by loss of nitrogen may give radical anions, and these (or other radicals derived thereof) may be capable of inducing DNA cleavage via radical mechanisms. (ii) Oxidation[4,6] (e.g., by metal ions) followed by loss of nitrogen may lead to radicals, and these (or other radicals derived thereof) may induce DNA cleavage via well established radical pathways.[7,8] These mechanistic possibilities, which give such an active role for the diazo group, are summarized in Scheme 1 using our first model compound 9-diazofluorene **3**. This, we believe, is justified because kinafluorenone,[9] a biosynthetic intermediate which lacks the diazo moiety, shows no activity.

Scheme 1. Potential DNA Cleaving Intermediates from 9-Diazofluorene 3



Due to our previous experience[1] in utilizing an *in situ* diazotization procedure (1.2 equiv of isoamyl nitrite, acetic acid, 25 °C, 30 min)[10] for DNA cleavage, we first sought to generate diazonium compound **4** (Scheme 1) directly from commercially available 9-aminofluorene. However, addition of this solution to aqueous buffers (pH = 4–7) containing the pBR322 supercoiled DNA did not produce any DNA cleavage; neither did the addition of cuprous chloride, which we had demonstrated to be successful in activating diazonium compounds for DNA cleavage.[1] A possible explanation is that, as an aliphatic diazonium ion, **4** may have decomposed to an inactive form prior to its addition to DNA.[4] Alternatively, under the diazotization condition, **4** may have been converted to 9-diazofluorene **3**; groups capable of conjugation, such as phenyl, are known to favor such transformations.[4]

Our second set of investigations began with the synthesis of 9-diazofluorene **3** from commercially available 9-fluorenone hydrazone (HgO, Et_2O followed by KOH in EtOH; yield = 91%).[11] The DNA cleavage experiments thus far performed may be summarized as follows: (i) In the pH = 4–7 range, we observed no cleavage. At pH < 4, control experiments reveal that destruction of the pBR322 supercoiled DNA was mostly acid induced. The contributions, if any, of intermediates derived from **3** could not be clearly determined. (ii) Cuprous chloride, which was successful in reductively activating diazonium compounds for DNA cleavage,[1] did not activate **3**. (iii) Efficient DNA cleavage was observed in the presence of cupric acetate.[6] The results of our experiments, as probed by 0.7% agarose gel electrophoresis, are shown in Figure 1. While no DNA cleavage was apparent with 9-diazofluorene **3** (lane 2) or cupric acetate alone (lane 7), the presence of both led to the cleavage of pBR322 supercoiled DNA (lanes 5 and 6). (iv) No DNA cleavage was apparent in the presence of other metal acetates, such as AgOAc (lane 3), $Tl(OAc)_3$ (lane 4), and $Hg(OAc)_2$ (lane 8), which are also known[6] to promote the decomposition of 9-diazofluorene **3**.

Encouraged by the results with 9-diazofluorene **3**, we next examined the DNA cleaving ability of β-naphthyl phenyl diazomethane **8** which mimics the ACD-rings of prekinamycin **2**.[11,12] Indeed, as indicated by the gel

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(12) Synthesis of **8** is described in the supplementary material.

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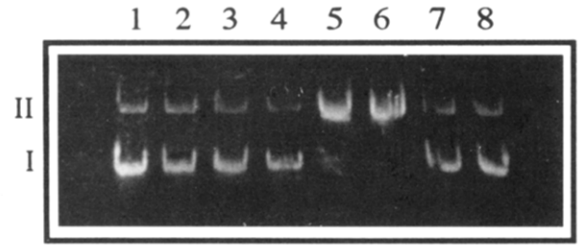


Figure 1. Cleavage of DNA by 9-diazofluorene **3**. All reactions, which were run for 24 h at 25 °C, contained 714 ng of pBR322 supercoiled DNA (64 nM) in a 10 mM Tris-HCl buffer, pH = 7.6, containing 5 mM NaCl and 0.1 mM EDTA. Electrophoresis was conducted at 50 V (2 h) on a 0.7% agarose gel, and the gels were stained with ethidium bromide after electrophoresis. Lane 1, control DNA; lane 2, DNA + **3** (500 μM); lane 3, DNA + **3** (500 μM) + AgOAc (1000 μM); lane 4, DNA + **3** (500 μM) + $Tl(OAc)_3$ (1000 μM); lane 5, DNA + **3** (250 μM) + $Cu(OAc)_2$ (500 μM); lane 6, DNA + **3** (500 μM) + $Cu(OAc)_2$ (1000 μM); lane 7, DNA + $Cu(OAc)_2$ (1000 μM); lane 8, DNA + **3** (500 μM) + $Hg(OAc)_2$ (1000 μM). Form I: supercoiled DNA. Form II: relaxed DNA (single-strand cleavage).

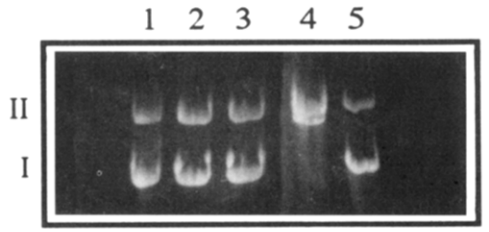
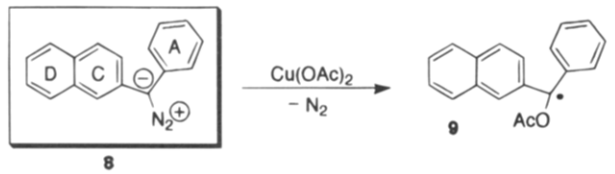


Figure 2. Cleavage of DNA by β-naphthyl phenyl diazomethane **8**. Reaction conditions, except for the concentrations mentioned here, are identical to those descibed in Figure 1. Lane 1, DNA + **8** (500 μM); lane 2, DNA + **8** (50 μM) + $Cu(OAc)_2$ (100 μM); lane 3, DNA + **8** (100 μM) + $Cu(OAc)_2$ (200 μM); lane 4, DNA + **8** (250 μM) + $Cu(OAc)_2$ (500 μM); lane 5, control DNA.

Scheme 2. β-Naphthyl Phenyl Diazomethane **8** for Inducing DNA Cleavage



(Figure 2), **8** also induced DNA cleavage in the presence of cupric acetate and this activity was essentially the same as that of 9-diazofluorene **3**.

What are the mechanisms associated with the aforementioned DNA cleavage reactions? Since all our experiments were performed in the dark, the involvement of carbenes[4,13] may be excluded in the cupric acetate-mediated DNA cleavage by 9-diazofluorene **3** (Scheme 1) or β-naphthyl phenyl diazomethane **8** (Scheme 2). However, contributions, if any, of copper-carbenoids[4,14] cannot be ruled out at this time. In a series of studies dealing with the metal-catalyzed decomposition of 9-diazofluorene **3**, Nozaki and co-workers[6] reported that cupric acetate in aqueous DMF provided the highest yield (~55%) of the fluorenone pinacol diacetate, the dimerization product of radical **7** (Scheme 1). In contrast, AgOAc, $Tl(OAc)_3$, and $Hg(OAc)_2$, the metal acetates that failed to activate 9-diazofluorene **3** for DNA cleavage, produced little or none of this dimerization product, and hence radical **7**.[6] Therefore, speculation that radical **7** (or in the case of β-naphthyl phenyl diazomethane **8**, radical **9**) may, in some way, be involved in the reactions that ultimately lead to DNA cleavage may not be unreasonable. Of the two possible scenarios, it is less likely that these stable, carbon-centered soft radicals will directly abstract hydrogen atom from the sugar-phosphate backbone of DNA; rather, this may be accomplished through the more reactive oxygen-centered radicals generated via the reaction of the carbon-centered radicals with molecular oxygen.[15,16] Alternatively, cuprous ions, produced during the oxidation of diazo compounds **3** (Scheme 1) and **8** (Scheme 2) by cupric acetate, may also generate active oxygen species, such as Cu(I)–oxygen complex or hydroxyl radical, capable of cleaving DNA.[16] While more work is needed to unequivocally determine the importance and/or contributions of each of the active species generated under our experimental conditions, the potential of the diazo group, and hence the kinamycin antibiotics, to cleave DNA in the presence of oxidants (e.g., cupric acetate) has been demonstrated. Since quinone, a potential oxidant,[17] is already built into the kinamycin structure, an internal triggering mechanism may be envisioned for the bioreductive alkylation events speculated for kinamycin C.[18] Our current studies are focused on addressing these particular issues.[19]

Supplementary Material Available: Experimental procedures and spectral data for the preparation of compounds **3** and **8** (2 pages).

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(19) This work was partially supported by the NSF (CHE-9406469).