Radical-induced DNA Cleavage Mediated by a Vinyl Epoxide

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Addition of thio radicals to a vinyl epoxide has resulted in formation of allyloxyl radicals which have cleaved DNA from ΦX174.

The mechanisms by which DNA can be damaged are currently the subject of intense interest.[1–3] Many anti-tumour agents work by alkylating DNA[2] while others destroy DNA by radical chemistry,[2] starting either (*i*) by abstracting a hydrogen atom from a deoxyribose sugar or (*ii*) by adding to the alkene π-bond in a base. Radical-induced DNA damage is also seen outside of chemotherapy, for example as a result of exposure to radiation. Hydroxyl radicals produced on radiolysis of water react with DNA by these two mechanisms also.[4]

The preceding paper has suggested an approach to treating tumours whose resistance to radiotherapy arises as a result of the protective effect of increased local glutathione (GSH) concentration. This approach features the activation of a vinyl epoxide by thio radicals. Encouraged by the success of these model studies, we now sought to effect DNA cleavage with a vinyl epoxide.

The compound chosen was the phenanthrolinium salt **1**, which was synthesised as shown in Scheme 1. On activation with a thio radical, the oxyl radical **2** would be produced and this oxyl radical should be very slow to add to the phenanthrolinium ring system, since both are electrophilic. Phenanthrolinium salts[5] have been widely used as DNA intercalators.

To make the test as biologically relevant as possible, the glutathionyl radical was used as the required thio radical and was generated by enzymatic[3] means using horseradish peroxidase. The incubation with DNA derived from ΦX174 led to the electrophoretogram shown in Fig. 1.

As seen in the electrophoretogram (lane 1), the commercial DNA from ΦX174 exists principally as the supercoiled closed circular duplex of form I, but with some form II present. Lane 2 represents a control to show that molecule **1** cannot effect DNA cleavage on its own under these conditions. It might have been assumed that **1** could act as an electrophilic alkylating agent, and that DNA cleavage would result. Lanes 3 and 4 show that neither glutathione nor hydrogen peroxide can, on their own, effect DNA cleavage. Lane 5 shows that under the established conditions for glutathionyl radical formation with horseradish peroxidase,[3a] but in the absence of **1** that no cleavage occurs. Lanes 6–8 demonstrate that DNA cleavage to the nicked form II and to a lesser extent to the linear form III occurs when the glutathionyl radicals and vinyl epoxide **1** are present. This shows that a combination of vinyl epoxides and thio radicals constitutes an efficient system for cleavage of DNA. In lanes 9–12, we investigate the exposure of DNA to tenfold lower concentrations of enzyme, of glutathione and of hydrogen peroxide. Under these conditions, we cannot see a noticeable difference between the control lane 9 in which no epoxide is present and the lanes 10–12 where the epoxide is present. This means that at these

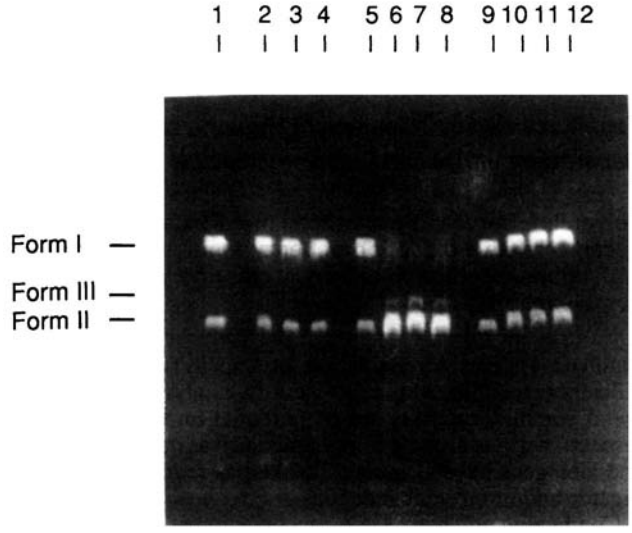
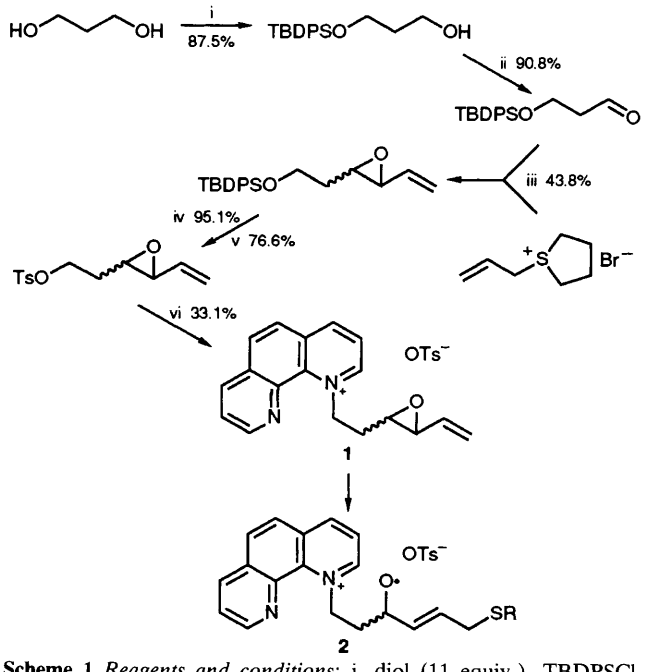
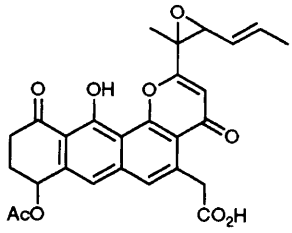


Fig. 1 Lane 1: DNA alone. Lane 2: DNA + 2.5 mmol dm^{-3} **1**. Lane 3: DNA + 100 mmol dm^{-3} GSH. Lane 4: DNA + 1 mmol dm^{-3} H_2O_2. Lane 5: DNA + 100 mmol dm^{-3} GSH + 1 mmol dm^{-3} H_2O_2 + 1 mg ml^{-1} horseradish peroxidase (HRP). Lane 6: as lane 5 plus 2.5 mmol dm^{-3} **1**. Lane 7: as lane 5 plus 250 μmol dm^{-3} **1**. Lane 8: as lane 5 plus 25 μmol dm^{-3} **1**. Lane 9: DNA + 10 mmol dm^{-3} GSH + 100 μmol dm^{-3} H_2O_2 + 0.1 mg ml^{-1} HRP. Lane 10: as lane 9 plus 2.5 mmol dm^{-3} **1**. Lane 11: as lane 9 plus 250 μmol dm^{-3} **1**. Lane 12: as lane 9 plus 25 μmol dm^{-3} **1**. Loading 0.5 μg DNA per lane (17 nmol dm^{-3}). All reactions were performed in 0.25 mmol dm^{-3} NH_4OAc buffer (8 μl, pH 7.05) at 25 °C for 1 h, under oxygen. Electrophoresis was performed on a 0.8% agarose gel at 100 V (3.7 V cm^{-1}) for 3 h (running buffer: 1 × TAE: 40 mmol dm^{-3} Tris acetate + 1 mmol dm^{-3} ethylenediaminetetraacetic acid, pH 8.2; ethidium bromide added).



Scheme 1 *Reagents and conditions*: i, diol (11 equiv.), TBDPSCl, imidazole, dimethylformamide (DMF), 2 days; ii, $(COCl)_2$, dimethyl sulfoxide (DMSO), Pr^i_2NEt, CH_2Cl_2, −65 to 25 °C, 2 h; iii, $BnEt_3NCl$, CH_2Cl_2, 10 mol dm^{-3} NaOH, −20 to 0 °C, 12 min; iv, Bu_4NF, tetrahydrofuran (THF), 2 h; v, TsCl, pyridine, 0 °C, 1.5 h; vi, 3.5 equiv. 1,10-phenanthroline, MeCN, 60–70 °C, 3 days (Ts = *p*-$MeC_6H_4SO_2$, TBDPS = *tert*-butyldiphenylsilyl)



Kapurimycin A3

concentrations, we are not able to detect cleavage of DNA. Cleavage is presumably still occurring but at a slower rate. This is reasonable since the concentration of enzyme of hydrogen peroxide and of glutathione have been dropped substantially, so a significant decrease in radical flux is expected. This demonstrates that a combination of vinyl epoxides and thio radicals constitutes a novel system for cleavage of DNA.

Recently, it has been shown that a natural vinyl epoxide,[6] kapurimycin A3, can cause DNA cleavage by electrophilically alkylating at N-7 of guanine; the results in this paper suggest than an alternative radical mechanism may also apply if the drug encounters naturally occurring glutathionyl radicals. We are currently exploring the *in vivo* activity of related vinyl epoxides.

It is also of interest to note the enzyme-driven DNA cleavage observed here marks the first use of a non-nuclease enzyme to cut DNA.

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