

Commentary

Possible Mechanisms for Strand-Breaks in DNA Induced by Stirring

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When aqueous solutions of DNA are stirred at room temperature, strand breaks occur extensively. Using various spin-traps, coupled with epr spectroscopy, we have shown that this does not proceed via homolysis. It is suggested that breaks occur by hydrolysis at strongly bent regions, momentarily induced by the stirring.

Keywords: DNA, Strand breakage, spin trapping

INTRODUCTION

Mechanical treatment of high polymers often results in homolytic bond-breaking.^[1,2] Recent examples are the homolytic breaking of the central collagen strands within bones, when the bones are fractured.^[3,4] Another is the formation of sulphur-centred keratin radicals when fingernails are cut.^[5] Stirring aqueous DNA is a well used technique for converting long strands into short strands. It therefore seemed a matter of interest to discover possible mechanisms for such breaks. So far as I am aware, no previous work has been done in this area.

It seemed possible that mechanical damage was involved, since stirring undoubtedly introduced strains of various sorts in macromole-

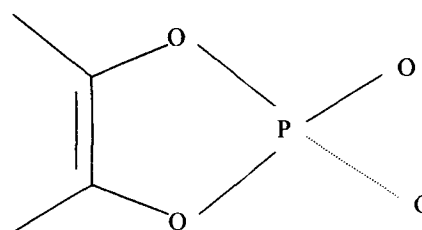
cules. These include bond stretching, and especially bond bending. Bond stretching could lead to homolysis thereby generating two active radicals. Accordingly, we stirred oxygen-free solutions obtained by passing pure nitrogen DNA through a diffuser for several hours in the presence of a range of spin-traps, including: 2-methyl-2-nitrosopropane, (NTB), 5,5-dimethylpyrroline N-oxide (DMPO), and phenyl-N-t-butyl nitron (PBN).

Even after prolonged stirring, thereby generating short strands, no epr signals could be detected. Had radicals been formed, relatively narrow epr features would have been detected because the radicals would be terminal and many of the strands would be short. In all cases, readily detectable concentrations should have been formed. Thus from studies of the epr spectra and the optical spectra of aqueous solutions of Fremy's salt, we showed that under our experimental conditions we were able to detect considerably less than 10^{-6} M of R_2NO radicals. Even so, we thought it would be of interest to use the method that we had previously developed to study other systems – namely, grinding under liq-

uid nitrogen. However, this also failed. Epr studies at 77K after extensive grinding, using dry DNA or frozen aqueous DNA, gave no detectable epr signals. Thus the concentration of radicals must be less than *ca.* 10^{-5} M. I conclude that bond homolyses are not involved in strand-breaks.

The only alternative that I can envisage is hydrolysis. In neutral solution this is, of course, an extremely slow process. However, it has been established, using compounds such as I and II (Figure 1), that bending at the phosphate site has a huge effect on the rate of hydrolysis, with large rate enhancements. I therefore propose that the very vigorous stirring that is involved causes distortions of this type that survive long enough to allow rapid addition of water to give hydrolysis. Westheimer and coworkers have found that five-membered cyclic esters (I and II) hydrolyse millions of times faster than their acyclic analogues.^[6-8] Thus, for example, they found that the rate of hydrolysis cyclic ethylene phosphate occurred at *ca.* 10^8 times faster than that of dimethyl phosphate. This was true at all pH values. However, 6- or 7- membered cyclic phosphates were hydrolysed at normal rates.^[9,10] The effect of ring strain greatly reduces the O-P-O bond angle, from *ca.* 109° to *ca.* 99° . This results in large increases in the rates of nucleophilic addition at phosphorus, which explains the increased rate of hydrolysis. In essence, this "opens" the site for a water molecule to add to the trigonal bipyramidal intermediate. That is all that is needed. The stresses involved in stirring can readily induce this relatively small degree of bending, and once water is added this can relax back without blocking the hydrolysis.

I therefore propose that the very vigorous stirring used to induce breaks causes bending, and that the bent units rapidly add water and hydrolyse. Some of this work was carried out at Leicester University by a variety of undergraduate chemists.



I, R=H II, R=CH₃

FIGURE 1 Structures of cyclic phosphates used by Westheimer (1968)^[6]

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