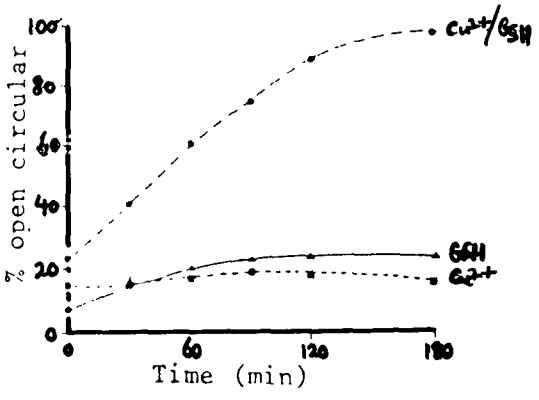
TOWARDS CHEMICAL CONTROL OF GENE EXPRESSION. COPPER(II) AND THIOLS, INCLUDING GLUTATHIONE, ARE POWERFUL REAGENTS FOR CLEAVAGE OF DNA

C.J. Reed[1] and K.T.Douglas, Department of Pharmacy, University of Manchester, Oxford Road, Manchester, M13 9PL and [1]School of Natural Sciences, Liverpool Polytechnic, Liverpool, L3 3AF

It is now reasonable to consider the development of synthetic molecules as drugs and tools for molecular biology based on DNA sequence-recognition. Such molecules would allow a particular gene sequence (e.g. a cellular oncogene) to be occluded and it is possible in principle to activate, suppress or modify gene activity by appropriate, sequence-directed ligands. Even more exciting is to combine sequence recognition with a chemical DNA-scission mechanism as has been so elegantly demonstrated using Fe:EDTA (Dreyer and Dervan, 1985), Cu:phenanthroline (Spassky and Sigman, 1985) or porphyrins (Lown *et al.*, 1986) as the cleaving agents. This paper gives details of the cleavage of plasmid DNA by an efficient combination, the Cu(II):thiol system.

In the presence of Cu(II) supercoiled DNA is cleaved in neutral solution by micromolar concentrations of a range of thiols including L-cysteine and glutathione (GSH). A time-course of the conversion of supercoiled to open circular DNA by GSH plus Cu^{2+} (final concentrations 5 and 100μM, respectively) is shown in the Figure. In the absence of either Cu^{2+} or GSH very little cleavage was seen. In the presence of various hydroxyl radical scavengers the following results were obtained (percent protection against supercoiled conversion to open circular is given in parentheses after the scavenger): 0.1M mannitol (0%); 10% glycerol (85%); 0.1M NaN_3 (100%); catalase at 1μg/ml (100%), boiled catalase at 1μg/ml (0%); superoxide dismutase at 1μg/ml (74%), boiled superoxide dismutase (78%).



The products of cleavage could be labelled by both the Klenow fragment of DNA polymerase 1 and by T4 polynucleotide kinase implying that at least some of the product DNA fragments bear 5'-OH and/or 3'-OH sites. Piperidine treatment of Cu(II):GSH cleaved plasmid DNA indicated that extensive base damage occurs under the Cu:RSH incubation conditions but that this does not necessarily lead to strand breakage. The attack of GSH plus Cu(II) on DNA may be a potential toxic lesion under physiological conditions unless special protective measures operate efficiently in the cell.

Dreyer, G.B., Dervan, P.B. (1985) Proc. Natl. Acad. Sci. (USA) 82: 963-967
Lown, J.W. *et al.* (1986) Biochemistry 25: 5111-5117
Spassky, A., Sigman, D. (1985) Biochemistry 24: 8050-8056