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PROTEIN-DNA-CROSSLINKS INDUCED BY PRIMARY AND SECONDARY RADICALS

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If DNA is γ-irradiated in the presence of bovine serum albumin (BSA) in a weight ratio 1 : 10, nearly all primary radicals are scavenged by the protein. The protein radicals can react with DNA by forming crosslinks. If radiolysis is carried out under anaerobic conditions and at low pH, crosslinking between protein and DNA is favoured above strand breaking of DNA.[1,2]

To find out whether only OH radicals induce crosslinking as was assumed previously,[3] or whether all primary radicals contribute to this reaction, the nitrocellulose filter assay was applied. This very sensitive filter assay is based on the fact that with rather high salt concentration protein is bound to the nitrocellulose filter while DNA passes through the filter. Since a single protein-DNA crosslink is sufficient to retain DNA on the filter, crosslinking can be measured already with doses below 100 Gy.

The filter assay confirmed our previous result that oxygen inhibits crosslinking. If hydrated electrons were converted to OH radicals by N_2O, there was no increase of crosslinking. If under these conditions 10^{-1} mol dm^{-3} ethanol was added, all OH radicals were scavenged so that only ethanol radicals remained, but still crosslinking was demonstrated by the nitrocellulose filter assay. If radiolysis of the same solution was carried out under N_2, the yield of crosslinks was increased. Using t-butanol as $^{\cdot}$OH-scavenger caused even higher yields of crosslinking. These results show that hydrated electrons and alcohol radicals can induce such protein radicals which react with DNA by crosslinking.

It is assumed that protein-DNA crosslinking is favoured if in the non-irradiated solution there is already a weak interaction between protein and DNA. This interaction will depend on the ionic conditions of the solution. If the concentration of the phosphate buffer is changed from 10^{-2} to 10^{-1} mol dm^{-3}, the electrostatic interaction between protein and DNA will decrease and consequently the yields of crosslinks are reduced.

References

1. Schuessler, H., Geiger, A., Hartmann, H. and Steinheber, J. The effect of a protein on the radiolysis of DNA studied by gel filtration. *Int. J. Radiat. Biol.*, **51**, 455, (1987).
2. Schuessler, H. and Hartmann, H. The effect of a protein on the radiolysis of DNA studied by HPLC and pulse radiolysis. *Int. J. Radiat. Biol.*, **52**, 269, (1987).
3. Oleinick, N.L., Chiu, S., Friedman, L.R., Xue, L. and Ramakrishnan, N. DNA-Protein Cross-Links: New insights into their formation and repair in irradiated mammalian cells. In *Mechanisms of DNA Damage and Repair*, Plenum Publishing Corporation, p. 181, (1986).