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Hydroxyl radical interference: a new method for the study of protein–DNA interactions

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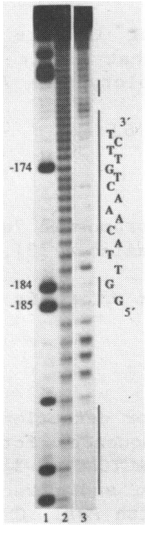
Understanding the interaction between DNA regulatory proteins and their cognate DNA sequences is a main goal in current attempts to unravel the mechanisms governing gene expression. Protection against hydroxyl radical attack at the sugar backbone detects intimate contacts between protein and DNA, independently of the nucleotide sequence. (1). The limitation is the need for relatively pure protein and high degree of DNA binding. However, if an interference version of the method is used in combination with a mobility shift assay, high resolution information can be obtained with less purified proteins or with low degree of DNA binding. In addition, this method yields information on the influence of single DNA nicks on protein binding.



A 57 bp fragment containing the promoter distal HRE of MMTV end-labelled with $^{[32]}P$ was modified with hydroxyl radicals as previously described (2). The reaction mixture, 200 µl, contained 10 mM Tris/HCl, pH 7.5, 0.2 mM dithiothreitol, 1.5% glycerol, 0.9 mM $(NH_4)_2Fe(SO_4)_2$, 1.8 mM EDTANa, 0.11 mM H_2O_2 and 9 mM Na-ascorbate. Following incubation at 25°C for 20 min, the reaction was stopped by addition of 45 µl 1.2 M NaOAc, 180 mM thiourea and 500 ng poly [d(A-T)]. After ethanol precipitation the DNA was incubated with partially purified progesterone receptor (4 ng) and applied to a 4% polyacrylamide gel (3). The free and retarded band were electroeluted, denatured and analyzed on a standard 15% polyacrylamide-8M urea gel. *Lane 1* shows a G-specific sequence reaction; *lane 2* shows the free DNA; *lane 3* shows the band retarded by the progesterone receptor. The protected regions and the relevant nucleotide sequence are shown.

The results agree with those of the footrpinting method (2) and show that nicking within the HRE at positions other than -183 is incompatible with receptor binding. Thus, the method is a valuable addition to the tools for analyzing protein-DNA interactions at the nucleotide level.

References:
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