Interaction of RNA polymerase T7 and carcinogen 4-nitroquinoloine-1-oxide [NQO] with DNA and effects on cleavage activity of restriction enzymes

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ABSTRACT

Gel Electrophoresis studies with DNA oligonucleotides have suggested that carcinogen 4-nitroquinoline-1-oxide [NQO] binds to DNA with sequence specificity and alters the enzymatic activity of restriction enzymes. Additionally, the reactivity of such enzymes is also altered through RNA polymerase T7 interaction with DNA. Studies suggested that T7, in the absence of promoter sequences, will slide through the DNA and bind to non-promoter sequences, affecting restriction enzyme activity. To test the occurrence and intensity of the effects of NQO and T7 on restriction activity, restriction enzyme assays were analyzed with increasing concentrations of NQO and a constant concentration of T7 via gel electrophoresis - beginning with the absence of both independent variables. NQO was added to DNA in a 0 to 4 NQO/DNA ratio and analyzed under simultaneous addition of T7 in a constant T7/DNA ratio of either 0 or 2. It is hypothesized that enzymes affected through NQO/DNA interaction will have similar motifs in flanking sequences, despite differing recognition sequences; and vice versa. Gel electrophoresis will specify whether the alterations witnessed resulted in either enhancement or inhibition of enzymatic activity and if they were concentration dependent. Similarly, for RNA Polymerase T7, it was hypothesized that T7 would have an affinity for sequences with a similar oligonucleotide sequence as that found in its original promoter sequence- 5’ – TAATACGACTCACTATAG – 3’. This study will reveal NQO and T7's ability to induce conformational changes in DNA which alter the enzymatic activity of specific restriction enzymes that contain specific motifs in their flanking or cleavage sequences.