Examining the effects of the binding of the carcinogen 4-nitroquinoline-1-oxide to DNA phiX174 on DNA structure using Mung Bean nuclease and Topoisomerase I assays

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Abstract

Optical titration studies, NMR studies and studies with DNA oligonucleotides have suggested that the carcinogen 4-nitroquinoline-1-oxide binds to DNA with marked sequence selectivity and cooperativity and that its binding affects the reactivity of enzymes with DNA. The observed cooperativity and enzyme effects suggest that the binding alters DNA structure. Using Mung Bean nuclease, an enzyme that recognizes regions of altered DNA structure, and Topoisomerase I, an enzyme which probes the unwinding of DNA, the binding effects of NQO to DNA phiX174 structure will be observed. To examine the effects of NQO on phiX 174, samples of supercoiled DNA are incubated with varying amounts and concentrations of NQO. Subsequently, the samples are reacted with either Mung Bean nuclease or Topoisomerase I and the reaction products are separated through gel electrophoresis. Interpretation of gel electrophoresis thus far shows enhancement of Mung Bean nuclease activity for DNA at [NQO]/[DNA bp] from 0.07 to 0.3, while exhibiting inhibition of Mung Bean nuclease for DNA at [NQO]/[DNA bp] from 0.7 to 3.0. Results for Topoisomerase I suggest that there may be some unwinding occurring in DNA phiX174. These results suggest that the binding of NQO to DNA alters the structure of DNA and provide insight into how NQO behaves as a carcinogen. Further analysis will be conducted in efforts of determining binding effects of NQO on DNA phiX174 using both enzymes. The use of enzymes in examining binding effects of highly carcinogenic compounds such as NQO on DNA suggest employing enzymatic activity is an efficient method to observe distortion of DNA structure. With these studies, the effects of the carcinogen NQO can be better understood and can contribute to studies attempting to combat the effects of NQO on DNA.