Abstract:

Locating high affinity binding sites of the carcinogen 4-nitro-quinoline-1-oxide on phiX174 DNA using restriction enzyme activity assays

Previous NMR and optical titration studies have suggested that the carcinogen 4-nitroquinoline-1-oxide [NQO] exhibits a high degree of selectivity in its binding to DNAs. Restriction enzyme activity assays have been employed to probe the sequence selectivity of a variety of compounds, including the carcinogen acetoxyacetylaminofluorene, to DNAs. To further examine sequence selectivity in the binding of NQO DNA, we have examined the binding of NQO to various locations on the sequenced DNA phiX174. In this study , several restriction enzymes, chosen to have different reaction sequences, have been used to examine the binding of NQO to the differing locations on the DNA phiX174. Samples of the DNA are reacted with varying concentrations of NQO and then digested with one of the restriction enzymes chosen to cleave phiX174 RF DNA once or twice. The digestion products are then separated on electrophoresis agarose gels. From the relative intensities of starting [undigested] DNA gel bands and digestion product gel bands enhancement or inhibition of cleavage by the bound NQO can be observed. The results of the digestions with the different enzymes are compared to detect sequence selectivity. For example, with the enzyme DraI [cleavage sequence TTTAAA], NQO showed enhancement of enzyme cleavage activity. In contrast, NQO produced inhibition at the enzyme XhoI site [CTCGAG]. The results of these experiments show that NQO shows selectivity in its binding activity on phiX174 DNA. When the sequence locations where this carcinogen binds to DNA are located, then we can examine the binding to those specific sequences in detail. These sequences can also be compared to the determined sequences for other carcinogens to provide understanding into how carcinogens, in general, act on the DNA.