125

Differential Effects on the Binding of the Transcription Factor, AP-1, to its Cognate DNA Sequence Containing the Nucleoside analog FIAU

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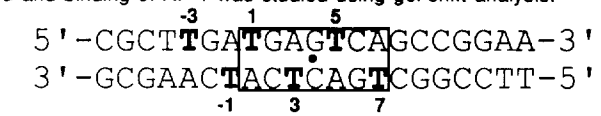
DNA-protein interactions are essential for many cellular processes such as genome replication and repair, recombination, and gene expression. The thymidine analog, FIAU, incorporates into nuclear, mitochondrial, and DHBV primer DNA. To study the effects of FIAU on DNA-protein interactions, we investigated the effect of incorporation of FIAU into DNA on the binding of a transcription factor. An oligonucleotide (below) which binds specifically to AP-1 was synthesized and binding of AP-1 was studied using gel-shift analysis.



The extent of binding of the above sequence to AP-1 was arbitrarily set to 100%. When thymidine at positions -3, -1, 1, or 7 was replaced with FIAU, binding to AP-1 was approximately 82%, 28%, 86%, and 51%, respectively, of the binding of the wild-type oligonucleotide to AP-1. When thymidine at position 3 or 5 was replaced with FIAU, binding of AP-1 was abrogated. Thus, when FIAU is used in place of thymidine at positions adjacent to the center of dyad symmetry (3 or 5) which are known to be important for the interaction with AP-1, binding was reduced dramatically. Consistent with these results, in competition experiments, oligonucleotides containing FIAU at positions 3 or 5 were less able to compete with radio-labeled wild-type oligonucleotide for binding to AP-1, suggesting a lower affininty for these FIAU-containing oligonucleotides. These results indicate that incorporation of FIAU into DNA does not alter the global structure of DNA, but may induce local conformational changes capable of disrupting specific DNA-protein interactions. These results indicate that the antiviral and/or cytotoxic properties of FIAU may be due, in part, to its ability to disrupt DNA-protein interactions.

126

Chain Terminated Hepadnavirus Genomes are Released as Complete Extracellular Virions: Are Serum Virion DNA Levels a True Measure of Drug Efficacy? B.E. Korba and J.L. Gerin. Division of Molecular Virology & Immunology, Georgetown University Medical Center, Rockville, MD USA.

The most common approach to therapy for HBV infection is the use of nucleoside analogues which terminate growing viral DNA strands. A fundamental issue pertinent to such treatments, the ultimate fate of chain terminated viral genomes, has not been adequately addressed. We have examined the characteristics of HBV and WHV virion particles produced by cultured human liver cells and chronic carrier woodchucks treated with 2,'3'-dideoxyguanosine (ddG). Both extracellular HBV virions and intracellular HBV core particles produced by 2.2.15 cells treated for 9 days with ddG had a significantly lighter buoyant density in CsCl, and a higher proportion of shorter DNA strands and single-stranded DNA, than HBV particles from untreated cultures. WHV virions from the serum of woodchucks treated with ddG for 4 weeks exhibited similar patterns of altered buoyant density and DNA content. By contrast, the physical characteristics of HBV DNA-containing virions and intracellular HBV particles from 2.2.15 cells treated with an antisense oligonucleotide (which would not act as a DNA chain terminator) were indistinguishable from HBV particles produced by untreated cells. HBV DNA in virions released by ddG-treated 2.2.15 cells also incorporated ^{3}H-labelled ddG, which was added to the cultures 48 hours prior to harvesting. The HBV and WHV genomes in intact virion particles produced following ddG treatment were contained in viral nucleocapsids which could be released by treatment of the virions with NP40 and ßME. Since a high proportion of the virion particles from ddG-treated cells contained apparently defective HBV genomes, we assessed the relative infectivity of these virion preparations in HepG2 cells using an *in vitro* infection method. Virion preparations from ddG-treated 2.2.15 cells were more than 100-fold less infectious than expected based on HBV genomic content. The infectivity of the WHV virion preparations in susceptible woodchucks is currently being evaluated. These results demonstrate that ddG-terminated HBV and WHV genomes are packaged, encoated and released as complete virions. Although, the quantitative measurement of hepadnaviral virion DNA levels will continue to be the most reliable indicator of antiviral activity, these data indicate that, depending on the antiviral agent used, a simple assessment of the levels of viremia may represent an underestimate of actual drug efficacy.