

NMR Structural Studies of Mass-Limited Modified DNA Using a Protasis/MRM CapNMR Probe

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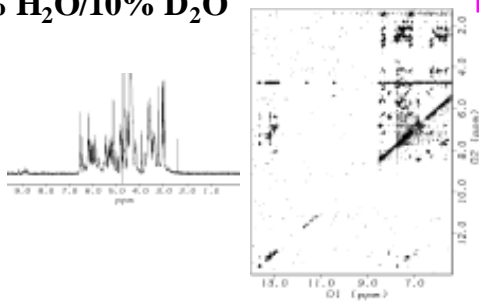
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Exposure to carcinogenic or mutagenic environmental agents often can lead to DNA base modifications that alter the structure and dynamics of the DNA. Possible outcomes, especially if the alterations involve regions important in maintaining proper cell growth and development, would be mutations, cancer, or cell death. While NMR structures and dynamics of many types of DNA lesions have provided detailed insights into the types of distortions that a cellular protein may encounter under biologically relevant conditions, the determination of the structures of numerous DNA adducts has been limited by the need to obtain milligram quantities of sample when using conventional 5 mm NMR probes. In several cases, it has been impossible or has taken several years to synthesize the sufficient quantities needed. The recent development of microcoil NMR detection technology (Protasis/MRM CapNMR probes) to enable high sensitivity detection of low abundance samples has allowed us to address this issue. An advantage of using oligonucleotide DNA is that it is highly soluble due to the negatively charged phosphate groups present, and thus microgram quantities can be readily concentrated into a very small volume without precipitation or aggregation. As proof of principle, we have compared the NMR data collected for a G/T mismatched 11mer duplex using a HCN, 5 μ l volume CapNMR probe with that obtained using a conventional Varian 5 mm, 600 μ l volume indirect H{N-P} probe. ¹H-1D, 2D-NOESY and phase sensitive COSY, and ¹³C-HMQC spectra were acquired on 12 mM (CapProbe) or 3 mM (5 mm Probe) sample dissolved in either 100% D₂O or 90% H₂O/10%D₂O 10 mM Na₂PO₄, 0.1 M NaCl, 0.1 mM EDTA buffer, pH 7.0 at 25 °C or 10 °C. The high quality of the data obtained with the CapProbe clearly demonstrates that structural studies of rare, mass-limited DNA adducts are now feasible.

5'-CCATATGCCC-3'
3'-GGTATATCGGG-5'

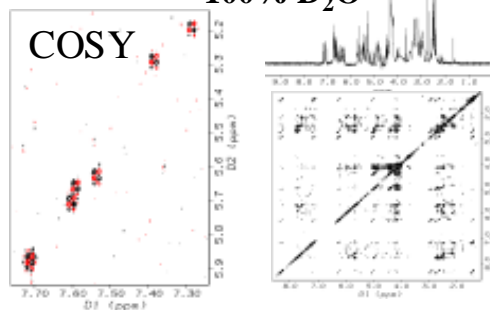
25 μ L of 6 mM guanine-thymine mismatch 11mer DNA in 20 mM Na₂PO₄, 0.2 M NaCl, 0.2 mM EDTA, pH 7.0 buffer.

90% H₂O/10% D₂O



150 ms mixing time NOESY

100% D₂O



300 ms mixing time NOESY

*Data collected with TXI CapNMR probe
Varian INOVA 600 MHz at 25°C*

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