Novel Isotopic Labeling Strategy for Nucleic Acids: Less is More

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Introduction

NMR spectroscopy has proven highly useful for structural characterization of macromolecules. However, large molecules exhibit numerous resonances which overlap one another and complicate studies. While protein isotopic labeling is readily available, labeling strategies for RNA and DNA can be cost prohibitive. A new procedure has been developed to both simplify DNA NMR spectroscopy and introduce new isotropic labels.

Methods

Synthesized DNA was dissolved in a solution of 50 mM trimethylol aminomethane pH 9 in 100% D$_2$O. The solution was then allowed to undergo exchange in a 60 °C water bath for one month. Upon completion of exchange, desalted and then lyophilized. Subsequently samples were prepared in H$_2$O or D$_2$O solutions as appropriate and studied by NMR spectroscopy.

Results

Monitoring the exchange kinetics of the H8 atom of adenine and guanine, the exchange for guanine was found to exchange faster. In addition the rate of exchange was pH dependent while adenine it was not. Following this, the aforementioned procedure was conducted using both a 10 mer duplex and G-quadruplex (Pu-22). When observed in water and D$_2$O solutions, both 1D and 2D NMR peaks involving H8 of guanine and adenine were greatly reduced or completely eliminated. Structural investigation via $^{31}$P and $^1$H imino NMR demonstrated that neither the backbone nor base pairing of the samples was perturbed. This confirms that our procedure does not affect the structure of the DNA samples.

Discussion:

The reduction and simplification of NMR spectra was accomplished by utilizing the exchange of GH8 and AH8 protons with deuterons. This can be achieved without disrupting DNA structure. Binding studies were therefore also facilitated. The rapid rate of guanine exchange specifically provides a timely and cost effective means for studying G-quadruplexes, specifically future work in more accurately studying grooves, loops, and flanking sequences.