Nucleoside containing a rigid nitroxide spin-label for structural studies of nucleic acids

Pavol Cekan, Nivrutti B. Barhate and Snorri Th. Sigurdsson
University of Iceland, Science Institute, Dunhagi 3, 107 Reykjavik, Iceland
pavol@mi.is

Abstract

The goal of this project was to design an efficient method for incorporation of a rigid nitroxide spin-label into nucleic acids to study the tertiary structure and dynamics of DNA and RNA by Electron Paramagnetic Resonance (EPR) spectroscopy.

We describe the completion of the first phase of this project, the synthesis of a nucleoside containing a rigid nitroxide spin-label and its incorporation into DNA. The spin-labeled nucleoside is an analogue of deoxycytidine, in which the nitroxide has been fused to the nucleoside base (**Figure 1)**. The nucleoside base-pairs to deoxyguanosine and therefore the spin-label is "fixed" relative to the DNA duplex. Thermal denaturation experiments and circular dichroism measurements have shown that the nucleoside has a negligible effect on duplex stability. EPR measurements of DNA oligomers containing the spin-labeled nucleoside showed that the nitroxide does not move independent of the nucleic acid. Furthermore, we have measured distances in DNA oligomers by continuous wave (cw) EPR and pulsed EPR methods. Reduction of the nitroxide functionality renders the spin-labeled nucleoside fluorescent. Thus, oligomers containing the new label can be studied by both EPR and fluorescence spectroscopy.

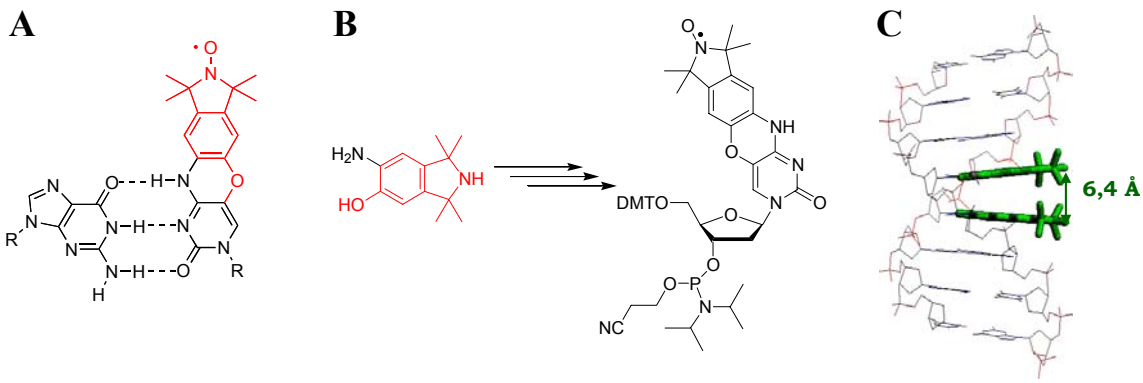

Figure 1A. *The deoxycytidine analogue rigid nitroxide spin label is shown base-paired to deoxyguanosine.* ***B.*** *Synthesis of spin-labeled phosphoramidite for incorporation of spin-label into DNA.* ***C.*** *The distance measured between two spin-labels (green, bold) within DNA duplex by EPR spectroscopy.*