Photophysical Studies with Fluorescent DNA Minor Groove Binding Agents

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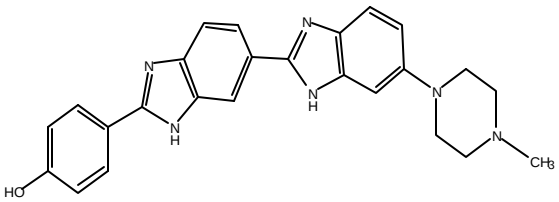
Given the central role of DNA in gene expression and regulation of biochemical processes, molecules that interact selectively with nucleic acids in a predictable way are of high interest in chemistry, biology, and medicine.

Using the fluorescent DNA minor groove binding agent, Hoechst 33258, as a model & reference, a series of analogues were designed and synthesized by Dr. M. P. Singh and Manashi Chatterjee, respectively, and were studied using fluorescence and other spectroscopic methods.

Absorption, emission, excitation and fluorescence quantum yield measurements (using quinine sulfate as a reference) were measured with the compounds dissolved in 10 mM phosphate buffer and 10 mM NaCl. Fluorescence lifetimes were also measured using laser spectroscopic methods.

Hoechst 33258



Hoechst 33258 exhibits an increase in fluorescence quantum yield of a factor of about 30 when bound to poly-A poly-T DNA. The analogues were studied and their molar extinction coefficients, quantum yields in buffer and in the presence of poly-A poly-T DNA were measured. Dimerization and/or precipitation of some analogues were observed.

Another set of analogues was designed to bind to sequences other than those rich in AT base pairs. These sequence-specific analogues were studied in a similar fashion as the other analogues. However, they were only weakly fluorescent and circular dichroism measurements were needed in order to prove binding was occurring.

The results will be discussed in terms of the structures of the fluorescent binding agents and their mode of binding to DNA.