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BINARY SOLVENT STUDIES OF HOECHST-33258

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Binary solvent studies of Hoechst 33258.

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Abstract

The dye Hoechst 33258 (a bisbenzimidazole derivative) becomes brightly fluorescent when bound to A-T rich sequences of double-stranded DNA. It is useful to stain chromosomal DNA for observation in a microscope; due to the virtual absence of fluorescence interference from dye bound to other cell components or from free dye, the chromosomes are very distinct. To better understand this behavior we have conducted both steady-state and time-resolved fluorescence measurements of Hoechst 33258 in binary solvent mixtures. We have found that the quantum yield of the dye is highly dependent on specific solvent interactions, and not on the bulk properties of the solvent. The time-resolved fluorescence data shows that Hoechst in solution has at least two species. One specie has a fast lifetime (<1 nsec) and a low quantum yield, the other specie has a slower lifetime (~3 nsec) and a much higher quantum yield. The long lifetime component is favored in non-aqueous environments. The sensitivity of the fluorescence properties to specific interactions is useful in model nucleotide studies where the bases are modified, providing information about the DNA-Hoechst interaction. (Partially supported by NIH grant RR03155).