Fluorescence lifetimes of nucleotide oligomers containing the cytosine analogue pyrrolocytosine

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Introduction

The natural nucleobases found in DNA: guanine (G), adenine (A), thymine (T) and cytosine (C) exhibit only extremely weak fluorescence. However, slight changes to the structure of the natural bases can lead to fluorescent compounds. Pyrrolocytosine (PC), shown in figure 1, is a fluorescent base structurally similar to the natural nucleobase cytosine and like cytosine pyrrolocytosine can base pair with guanine in double-stranded DNA. As the fluorescence of pyrrolocytosine is quenched upon helix formation the base can be used as a marker for the local structure and dynamics of nucleic acids.[1]

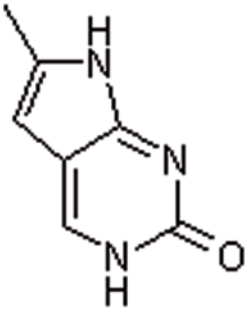

Figure 1. Structure of pyrrolocytosine, PC.

The mechanism by which the fluorescence of pyrrolocytosine is quenched upon helix formation is not known, although it is likely that the increase in base-stacking that occurs with helix formation, the formation of hydrogen bonds to the complementary base (guanine), or a combination of both enhanced base-stacking and hydrogen-bonding is responsible. In this work we have used time-correlated single photon counting to measure the aqueous-phase excited-state lifetimes of a series of di- and trinucleotides containing pyrrolocytosine, in order to determine the effect of base stacking on the fluorescence of pyrrolocytosine.

Experimental

Fluorescence decay curves for the following species in 50 mM pH7 phosphate buffer solution were obtained as a function of temperature (15 - 65°C): pyrrolocytosine, PC, the corresponding nucleoside, PC_{dr}, the nucleoside triphosphate, PC_{tp}, the dinucleotides PC-T, PC-C, PC-A and PC-G, and the trinucleotides T-PC-T and G-PC-G. Samples were filtered through a sterile Pall Acrodisc 0.2 µm Supor membrane syringe filter before use. The sample, housed in a temperature regulated quartz cuvette, was excited using 350 nm light with a pulse width of 180 fs. Light of 460 and 500 nm, emitted at 90° to the incident beam, was detected as a function of time using time-correlated single photon counting. The 350 nm incident light was obtained by frequency doubling the output at 700 nm from a mode-locked Coherent MIRA 900 Titanium:Sapphire laser operating at 75 MHz. The instrument response function was measured daily and found to be ~40 ps.

Results and Discussion

The fluorescence lifetimes obtained at 25°C are shown in Table 1.

Compound	Fluorescence lifetime / ns
PC	2.9
PC_{dr}	2.1
PC_{tp}	2.1
$^{5'}$PC-T$^{3'}$	2.0
$^{5'}$PC-C$^{3'}$	2.5
$^{5'}$PC-A$^{3'}$	2.7
$^{5'}$PC-G$^{3'}$	2.0
$^{5'}$T-PC-T$^{3'}$	3.0
$^{5'}$G-PC-G$^{3'}$	0.65 and 2.6

Table 1. Fluorescence lifetimes for compounds in pH7 buffer at 25°C.

In all cases except that of the G-PC-G trinucleotide the fluorescence decay curves obtained fitted well to a single exponential. In the case of G-PC-G it was necessary to fit a double exponential to get a good χ^2 value. Decay curves obtained in the case of the trinucleotides T-PC-T and G-PC-G are shown in figures 2 and 3 respectively.

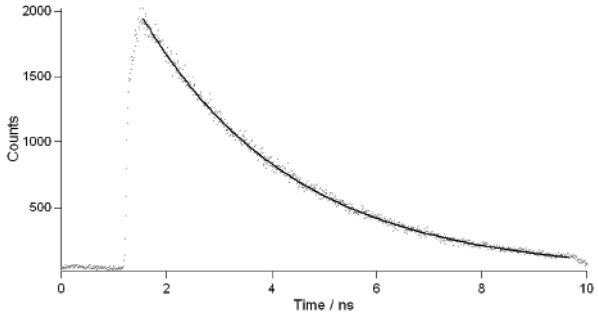

Figure 2. Fluorescence decay curve obtained for the trinucleotide T-PC-T. A monoexponential function has been fitted to the data (solid line).

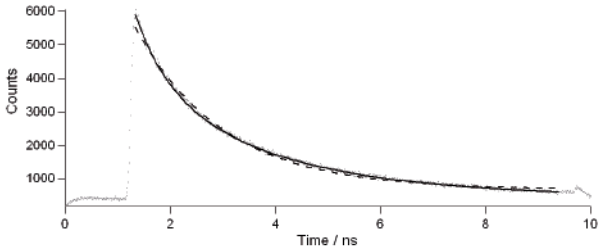

Figure 3. Fluorescence decay curve obtained for the trinucleotide G-PC-G. A biexponential function has been fitted to the data (solid line). Fitting a monoexponential (dashed line) resulted in a poor fit.

In the case of the G-PC-G trinucleotide the results indicate that the fluorophore exists in one of two possible environments. In one environment the fluorophore has a longer fluorescence lifetime than the free pyrrolocytosine nucleotide, possibly due to enhanced solvent shielding provided by the neighbouring guanine moieties. In the other environment the fluorophore has a significantly shorter fluorescence lifetime than the free base. It is thought that a particular base-stacked environment of pyrrolocytosine between two guanines must exist that facilitates a rapid nonradiative pathway, possibly involving transfer of an electron from the guanine (the most readily oxidised of all the natural nucleobases) to the pyrrolocytosine moiety.

Conclusions

The results obtained demonstrate that the fluorescence quenching that occurs when single-stranded nucleic acids containing the fluorescent cytosine analogue pyrrolocytosine hybridise to form a double helix is not caused purely by a base-stacking interaction with the neighbouring bases, unless the neighbouring bases include a guanine moiety. This results is in contrast to that found for the fluorescent adenine analogue 2-aminopurine, where incorporation into di- and tri-nucleotides containing any of the natural nucleobases has been shown to significantly reduce the fluorescence lifetime.[2]

References

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