

Photophysical processes of the spectroscopic RNA probe 2-(1-ethynylpyrene)-adenosine (PyA)

P. Trojanowski¹, J. Plötner¹, C. Grünewald², M. Braun¹, A. Reuss¹, J. Engels² and J. Wachtveitl¹
¹*Institut für Physikalische und Theoretische Chemie* and ²*Institut für Organische Chemie, Johann Wolfgang Goethe-Universität, Max-von-Laue-Str. 7, 60438 Frankfurt am Main, Germany*
E-Mail: wweitl@theochem.uni-frankfurt.de

Abstract. We examine the photoinduced excited state dynamics of pyrene modified adenosine, a versatile probe for folding and hybridization of ribonucleic acids. Measurements in different solvents revealed complex ultrafast dynamics, but high robustness since the overall fluorescence quantum yield (Φ_f) is hardly affected. The result is a strong fluorescent RNA-probe whose spectral properties change in a defined way upon environmental changes.

1 Introduction

Due to its high fluorescence quantum efficiency and the sensitivity on its environment pyrenes are commonly used as fluorescent probes for/in biomolecular spectroscopy [1-3]. Pyrene modified adenosine (PyA, Fig. 1, A) represents a fluorophore label that can e.g. act as an universal RNA folding probe which only slightly disturbs the RNA structure. The pyrene moiety is thought to point out into the minor groove of RNA in an A-form helix [4]. Another favorable characteristic is the ability to form excimers upon close proximity of two PyAs of complementary strands in the minor groove [4]. Experiments revealed that PyA is also able to intercalate into RNA depending on the flanking bases in 5' direction of the modified base [5, 6]. A G-C base pair leads to the intercalation of PyA, whereas A-U base pairs do not show this behavior. To get a further understanding of the processes, the photophysical properties of PyA were investigated with femtosecond pump-probe absorption spectroscopy and time correlated single photon counting (TCSPC) [6, 7]. PyA showed the existence of a locally excited (LE) state with sequence dependent lifetimes around 400 ps and presumably a relaxed S1 state with a lifetime around 2 ns. These states differ only slightly in their energy and interconvert on a timescale of 3 ps [6]. The above mentioned excimer shows an additional lifetime of approximately 19 ns and a spectral red-shift of 50 nm. Here we investigate the dependence of PyA on the solvent polarity. Combining these results with DFT calculations we are able to obtain a better understanding of the occurring processes. We observed a high sensitivity of PyA to its surroundings, but robustness of the high quantum yield. This makes PyA a suitable probe for more complex RNA systems.

2 Materials and Methods

PyA synthesis is described in [4]. The PyA solutions were prepared by dilution of a PyA DMSO stock solution with the dried solvents. The end concentration of DMSO was approximately 2.5 %. No change in the absorption or fluorescence spectra in comparison to solutions without DMSO was observed. The pump-probe absorption spectroscopy setup was described in detail elsewhere [8]. The samples were excited with the frequency doubled pulses of a CLARK CPA 2001 (Clark-MXR, Dexter, MI), providing excitation laser pulses of 150 nJ at 388 nm with a pulse duration of 200 fs. UV-vis spectra recorded before and after the measurements revealed only slight photodegradation during the measurements. For the data analysis the solvent background along with the coherent artifact was subtracted and corrected for group velocity dispersion [9]. The quantitative analysis was performed employing a kinetic model describing the data as a sum of exponential decays [7].

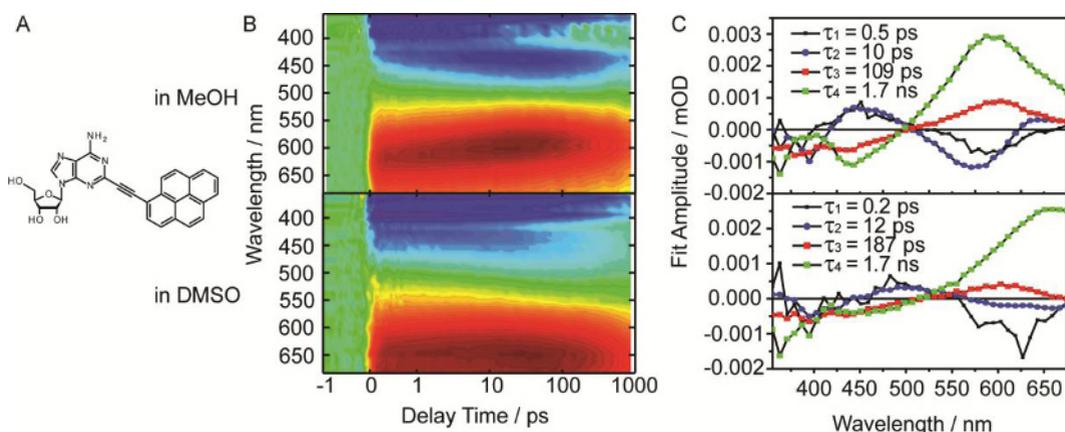


Figure 1: Structure of 2-(1-ethynylpyrene)-adenosine (PyA) (A). Transient absorption difference spectra (B) and decay associated spectra (C) of PyA in MeOH (top) and DMSO (bottom), τ_1 - τ_4 denote time constants (in ps) derived from the global fitting procedure.

3 Results

The transient absorption spectra (Fig. 1, B) show the evolution of the electronic states after excitation of PyA in MeOH (upper panel) and DMSO (lower panel) over a time range of 1 ns in the spectral range from 355 to 675 nm. Those spectra were taken for a total of 4 protic and 5 aprotic solvents (data not shown) covering a broad range of polarity. The spectral characteristics in MeOH are similar to those reported for PyA introduced in RNA strands dissolved in buffer [7] and of a silyl-protected derivate of PyA in MeOH [3]. A global analysis revealed that four time constants are necessary for a sufficient description of both data sets. τ_1 is somewhat ambiguous since it also contains coherent contributions of the solvent and is therefore not discussed any further.

PyA in MeOH shows a blue-shift of the excited state absorption (ESA, 620 to 600 nm; Fig. 1, B and C) and a corresponding red-shift of the stimulated emission (SE, 430 to 450 nm) with a time constant τ_2 of 10 ps indicating a relaxation process on the S_1 potential energy surface. A solvent with lower polarity e.g. tBuOH, slows down this process from 10 ps to 155 ps. DMSO and all other aprotic solvents show a lack of this spectral shift of the ESA and SE, but exhibit a population which rises with a time constants τ_2 in the same time range (12 ps in DMSO, 575 nm). This leads to the conclusion that protic solvents interact with PyA via a specific hydrogen bond but only stabilize a state already existing in aprotic solvents. This state decays with τ_3 (109 ps in MeOH and 187 ps in DMSO) to the final fluorescent state.

In aprotic solvents τ_3 leads most likely to the shift of the SE around 450 nm which is the result of the faster decrease in intensity of this closely lying electronic state with an ESA at 575 nm, which overlaps with the SE at early times. This state remains at this point not assignable, a TICT state as

well as rotational diffusion were ruled out by quantum chemical calculations and magic angle measurements, respectively.

The remaining time constant τ_4 (1.7 ns in MeOH and DMSO) describes the decay of the electronic state which dominates the overall spectra. The τ_4 decay is dominated by the ESA signal (protic solvents: ~ 600 nm; aprotic solvents ~ 650 nm). The decay associated spectra indicate that PyA shows similar ultrafast dynamics in both classes of solvents but differ slightly in the reaction rates and stability of each electronic state.

4 Discussion

In accordance to other pyrene derivatives PyA reaches the S_1 state within our time resolution ≤ 200 fs described by τ_1 . The τ_2 and τ_3 time constants cannot be assigned to specific electronic states, whereas τ_4 definitely describes the repopulation of the ground state.

In protic solvents the spectral blue shift describes most likely a stabilization of the electronic potential surface on a time scale of approximately 10 ps due to solvent interactions. This also indicates the existence of a hydrogen bond between PyA and the solvent since these processes are seen only in protic solvents. The process becomes more efficient with increasing solvent polarity as reflected by the overall faster time constants.

PyA in aprotic solvents exhibits the same time constants, but the spectra are dominated by the LE state absorption. Also the stabilized electronic state is depopulated on the same time scale (100 to 200 ps) and lies in the same spectral region as in protic solvents. The fact that PyA has a strong fluorescence as well as similar life times (~ 1.5 ns) is in line with similar oscillator strengths obtained by DFT methods (f_{osc} (unprotonated PyA) = 1.04 and f_{osc} (protonated PyA) = 1.05) for both solvent classes.

In summary, PyA fluorescence characteristics are influenced by the solvent type and therefore by environmental changes, but these changes only weakly affect the overall fluorescence quantum yield. Introducing PyA into RNA structures therefore provides a direct observable for the structural (re-)arrangements and conformational changes of RNA with a high quantum yield fluorophore. To gain more insight into the molecular mechanisms of PyA photochemistry further DFT calculations will help to verify the energetically possible positions of the hydrogen bond and to explain the similarity of both species. An alternative approach for structural surveys will be tested by the linkage of adenosine at position 1 of pyrene which should show a weaker electronic coupling [10] and which possibly reduces the intercalation of PyA with the RNA. Currently other approaches like new chromophores and FRET-pairs as conformational probes for RNA are tested.

5 References

1. J. Huang, Y. Wu, Y. Chen, Z. Zhu, X. Yang, C. J. Yang, K. Wang, and W. Tan, *Angew. Chem. Int. Ed.*, **50**, 401-404 (2011).
2. N. Venkatesan, Y. J. Seo and B. H. Kim, *Chem. Soc. Rev.*, **37**, 648-663 (2008).
3. C. Wanninger-Weiß, L. Valis and H.-A. Wagenknecht, *Bioorg. Med. Chem.*, **16**, 100-106 (2008).
4. C. Grünewald, T. Kwon, N. Piton, U. Förster, J. Wachtveitl and J. Engels, *Bioorg. Med. Chem.* **16** (1), 19-26 (2008).
5. U. Förster, K. Lommel, D. Sauter, C. Grünewald, J. W. Engels and J. Wachtveitl, *ChemBioChem*, **11**, 664-672 (2010).
6. U. Förster, C. Grünewald, J. W. Engels and J. Wachtveitl, *J. Phys. Chem. B* **114**, 11638-11645 (2010).
7. U. Förster, N. Goldenhoff, C. Grünewald, J. W. Engels and J. Wachtveitl, *J. Lumin.* **129**, 1454-1458 (2009).
8. M. O. Lenz, R. Huber, B. Schmidt, P. Gilch, R. Kalmbach, M. Engelhard and J. Wachtveitl, *Biophys. J.* **91**, 255-262 (2006).
9. S. A. Kovalenko, A. L. Dobryakov, J. Ruthmann and N. P. Ernstring, *Phys. Rev. A* **59**, 2369-2384 (1999).
10. A. G. Crawford, A. D. Dwyer, Z. Liu, A. Steffen, A. Beeby, L. Palsson, D. J. Tozer and T. B. Marder, *J. Am. Chem. Soc.* **133**, 13349-13362 (2011).