Influence of Intramolecular Hydrogen Bonding on the Photodynamics of 2-(1-Ethynylpyrene)-Adenosine (PyA)

P. Trojanowski[1], C. Grünewald[2], F. F. Graupner[3], M. Braun[1], A. J. Reuss[1], J. W. Engels[2] and J. Wachtveitl[1]

[1]Institute of Physical and Theoretical Chemistry and [2]Institute of Organic Chemistry and Chemical Biology, Johann Wolfgang Goethe-Universität, Max-von-Laue-Str. 7, 60438 Frankfurt a. M., Germany. [3]Faculty of Physics, Center for Integrative Protein Science, Ludwig Maximilians University Munich, Oettingenstrasse 67, 80538 Munich, Germany
E-Mail: wveitl@theochem.uni-frankfurt.de

Abstract: We report on the influence of intramolecular hydrogen bonding between the 2'OH group of ribose and adenine in 2-(1-ethynylpyrene)-adenosine (PyA) on the ultrafast dynamics, by comparing PyA with its deoxy derivate (PydA).
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1. Introduction

Pyrene-modified molecules are commonly used as versatile fluorescent probes for studies of biomolecular systems like RNA, proteins and membranes [1–3]. We designed the 2-(1-ethynylpyrene)-adenosine (PyA, Fig. 1A) as a fluorescent probe for RNA dynamics [4–7]. Its high fluorescence quantum yield as well as its high sensitivity to environmental changes, especially to the flanking bases [7], makes it an excellent probe for hybridization studies and conformational changes, e.g. due to ligand binding.

Femtosecond transient-absorption spectroscopy and time-correlated single-photon counting (TCSPC) revealed quite complex ultrafast dynamics [5,6]. These dynamics are influenced strongly by intramolecular hydrogen bonding. Herein we present our results on the influence of the 2'OH group on the photo-physical processes of pyrene modified adenosine (PyA) by comparison to the pyrene modified deoxyadenosine (PydA, Fig. 1B&C).

2. Material and Methods

2-(1-ethynylpyrene)-adenosine (PyA) and -deoxyadenosine (PydA) (Fig. 1A) were synthesized using methodologies described earlier for the synthesis of protected PyA [4].

The time-resolved transient-absorption (TA) measurements (Fig. 1C) were performed on a self-built pump-probe setup [8]. The samples (150 to 200 µM) were excited with the frequency-doubled pulses of a CLARK CPA 2001 (Clark-MXR, Dexter, MI), providing excitation pulses at 388 nm (150 nJ, 200 fs). TA measurements were corrected for solvent background and coherent artifacts and subjected to a lifetime density analysis (LDA, Fig. 1D). The pre-exponential amplitudes are determined in a sum of a large number (>50, typically ~100) of exponential functions with fixed lifetimes. The LDA is, unlike a global lifetime analysis, model independent and is able to describe non-exponential or stretched exponential kinetics [9].

3. Results

PyA and PydA show a complex time-resolved behavior in DMSO (Fig. 1C). Four time constants are required to analyse both PyA [5] and PydA (Fig. 1D). The fastest component (τ_1) is in the sub picosecond time range and due to the IVR of the S_1 state (excited state absorption (ESA1), 667 nm).

The second time constant (τ_2) describes the population of a second conformational state S_{1x} (ESA2, broad shoulder at ~625 nm) with a higher dipole moment than the S_1 state. The time constant for the population of the S_{1x} state (τ_2) for PyA and PydA is fairly similar with 3.7 ps and 2 ps, respectively. Both observed states are strongly emitting. Therefore, they contribute to the stimulated emission (SE) between ~400 nm and ~525 nm (Fig. 1C). The corresponding SE1 and SE2 bands are directly connected to the respective ESA1 and ESA2 signals. The SE1 band dominates the SE in the case of PyA, while the visible only at early times for PydA. On the other hand, the SE2 band dominates the spectrum in PydA and no significant contributions to the SE are observed at longer delay times.

The τ_3 component is mainly caused by the dynamic spectral shift of the SE2 band. With ~40 ps, it is completed much slower for PyA than for PydA (<10 ps). The population of the S_{1x} state occurs in the same time range as this spectral shift.

The ground state is populated from both states (S_1 and S_{1x}) with τ_4. The exact values were determined with streak-camera measurements (data not shown) and are 2.1 ns and 2.6 ns for PyA and PydA, respectively.

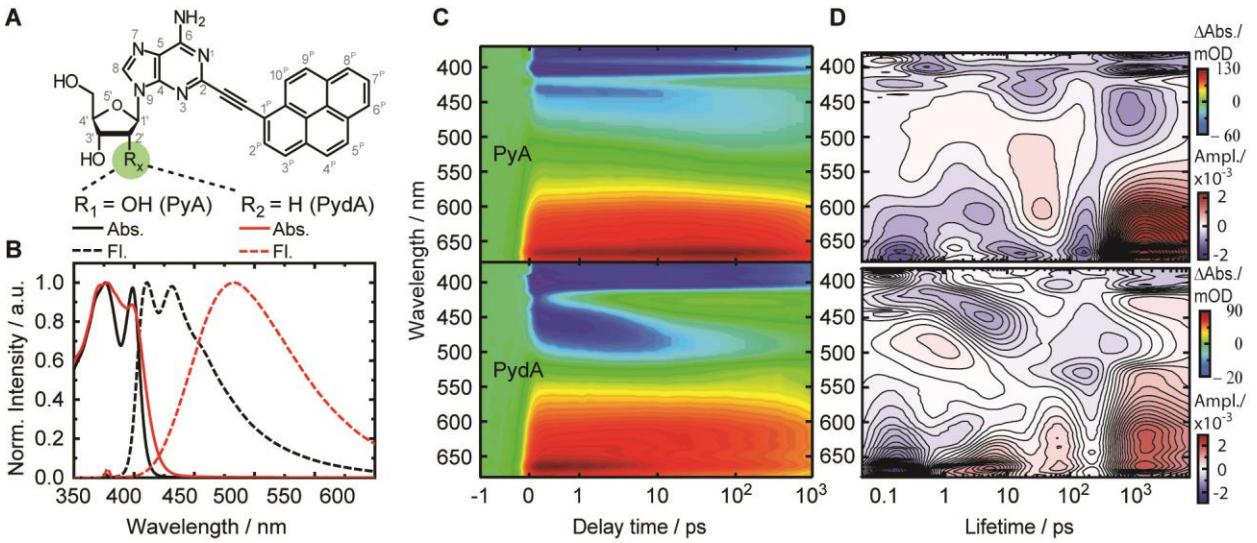

Fig. 1: PyA and PydA (**A**) as well as steady-state absorption an d fluorescence (λ_{exc} = 376 nm and 378 nm for PyA and PydA , respectively) spectra in DMSO (**B**) with the corresponding transient absorption spectra (371 nm to 679 nm, λ_{exc} = 388 nm) in DMSO (**C**), along with the LDA maps (**D**).

4. Discussion

Substitution of the 2'OH group of the ribose with hydrogen favors the population of the S_{1x} state with respect to the S_1 state, due to a lower energy barrier between the S_1 and the S_{1x} state. Additionally, the spectral shift of the S_{1x} state is strongly accelerated due to the missing intramolecular hydrogen bonding and the polar character of the S_{1x} state. Taking into account the steady-state measurements (data not shown) the spectral shift increases gradually with increasing solvent permittivity. In combination with the fact that the 2'OH group is too far away to interact directly with the conjugated electron system of pyrene, we can assume that hydrogen bonding of the 2'OH group with the adenine is responsible for these observations. The only accessible position is the N3 of the adenine, due to its proximity to the 2'OH group (Fig. 1A). The result of the hydrogen bonding is a reduced energy barrier for the $S_1 \rightarrow S_{1x}$ transition, due to an altered electron density on the N3. In combination with the overall faster spectral shift (Fig. 1C), this indicates that the altered electron density either increases the polar character of PyA or a steric hindrance is the reason for the higher energy barrier in case of PyA .

5. References

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