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Orientation-dependent quenching of the triplet excited 6-thiopurine by nucleobases

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ABSTRACT

Nanosecond transient absorption and steady-state photochemical studies showed that interactions of the nucleic acid bases: uracil, thymine (5-methyluracil), 5-ethyluracil and adenine with triplet excited 9-substituted 6-thiopurine chromophore (TP) is influenced by the mutual orientation of the heterocyclic rings. In an aqueous solution containing a mixture of monomers, where no restrictions are imposed on ring-to-ring orientation, all the nucleobases quench the T_1 state of 9-propyl-6-thiopurine (PTP) with similarly large rate constants ($k_q^{inter} \sim 10^9\ M^{-1}\ s^{-1}$). Steady-state irradiation of TP in the presence of uridine and adenosine led to adducts formed via [2 + 2] cycloaddition of C=S to the olefinic fragments of the nucleobases. In newly synthesized dyads composed of trimethylene-linked TP and nucleobase pairs, only thymine and 5-ethyluracil reduced the TP-like T_1 state lifetime ($k_q^{intra} \sim 5 \times 10^6\ s^{-1}$). The relative orientations of the 6-thiopurine and nucleobase rings in the dyads are limited by the spacer. The length of the trimethylene chain does not allow for a close approach of the reactive centers for [2 + 2] photocycloadditions. In steady-state irradiation only the dyads containing thymine or 5-ethyluracil are photoreactive, and they form intramolecular cyclophane-type products albeit with low quantum yields ($\phi \leq 6 \times 10^{-3}$). The structure of the photoproducts can be rationalized by assuming an initial H atom abstraction from the 5-alkyl group at the C(5) position of the uracil ring by an excited thiocarbonyl group. The preferential reversal of biradicals formed from [2 + 2] photocycladditon and from H atom abstraction has been suggested as mechanisms responsible for quenching of the TP T_1 state by nucleobases.

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1. Introduction

Utilizations of 6-thiopurine for structural studies of nucleic acids [1–3] and as a potential phototherapeutic agent [4,5] explore its photochemical reactivity in polynucleotides labeled with this modified, sulfur containing purine base. The 6-thiopurine chromophore absorbs at longer-wavelengths than do common, non-sulfur containing purine and pyrimidine nucleic acid bases. The thiocarbonyl group containing purine is therefore the primary excitation target upon UV-A irradiation of the biopolymeric systems containing it. Despite the importance of the photoreactions initiated by excited 6-thiopurine, the electronically excited states of the compound in such assembled systems have not been hitherto observed directly, and the dynamics of their depopulation have not been determined. The spectral, photophysical and photochemical studies have been confined to monomeric species: 6-thiopurine [6] in organic solvents, 6-thiopurine 2′,3′,5′-tri-O-acetylriboside (TI) in acetonitrile and in aqueous solution [7,8]. It has been found that the S_2 (π,π^*) state directly formed by irradiation within an intense absorption band (e.g.: TI in CH_3CN λ_{max} = 325 nm, ε_{max} = 22,500 $M^{-1}\ cm^{-1}$ [7]) undergoes efficient and fast relaxation to the lowest T_1 (π,π^*) state ($\phi_T = 1$ [6]).

Extrapolations of the photophysical characteristics of a monomer to a polymeric system may not be, however, appropriate. Indeed, a number of early as well as recent papers on the photophysics of non-modified bases containing dinucleotides, oligonucleotides and more structurally complicated related systems have demonstrated that the deactivation channels of the singlet excited state reached directly by the light absorption are altered in oligomers. This is due to the interactions such as base–base stacking in the polymer and, possibly, to the formation of H-bonded base pairs in nucleic acids duplexes [9–12]. As a consequence, the electronic nature and reactivity of the excited state involved in photochemistry may be changed upon incorporation of the monomer into a biomacromolecule [13]. The results of the very recent femtosecond time-resolved studies of several

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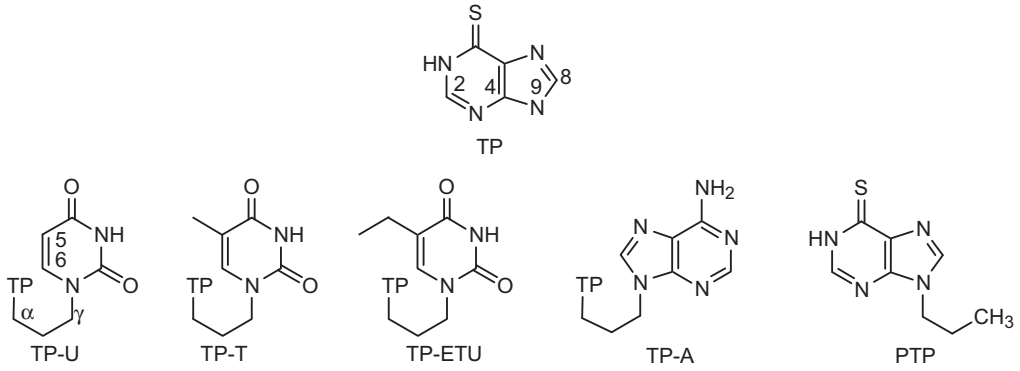

Chart 1. Structures of the compounds and atom numbering.

diribonucleoside monophosphates ApA, ApG, ApC, ApU, and CpG have been considered as evidence for the formation of singlet exciplexes with lifetimes much longer (ps timescale) than those of the singlet excited states of the monomers (fs timescale) [11]. Because of the fundamental relevance of the photophysics of nucleic acid to an understanding of the mechanism of the UV induced photodamage in living systems, most studies have been performed on shorter or longer biopolymer fragments composed of canonical purine and pyrimidine nucleosides. The photophysics of oligonucleotides containing thiocarbonyl derivatives of the nucleobases have received very little attention. To our knowledge, only a single report, concerning the photophysics of tRNA's containing the 4-thiouracil riboside (TUrd), has appeared [14]. The T_1 (π,π^*) excited state of TUrd is the photoreactive state both in photoreactions of tRNA and in solutions of the monomers. The estimated intersystem crossing yield for TUrd in tRNA is ~1/3 of that determined for monomeric 4-thiouridine ($\phi_T = 1$ [2]). The triplet lifetime of TUrd increases when it is in tRNA as compared to the free monomer in solution. The rigidity of the structure has been suggested as a possible reason for the ϕ_T decrease and the T_1 state lifetime lengthening when TUrd is inserted into tRNA [14].

In this paper we report the results on our study of a series of a newly synthesized TP-nucleobase dyads using nanosecond laser flash photolysis (LFP) and steady-state photochemistry. The dyads are simple models of dinucleotides, and they are composed of covalently linked 6-thiopurine and a common nucleobase (uracil, thymine, adenine) or 5-ethyluracil, a system of relevance with respect to its potent antiviral properties [15]. The structures of the compounds and the acronyms used throughout this paper are presented in Chart 1. All studies were performed in biologically important aqueous media. The results obtained for the dinucleotide models are discussed in relation to the monomeric chromophores.

2. Experimental

2.1. Materials

Synthesis of 9-propyl-6-thiopurine (PTP), TP-T and 1-propyluracil, 1-propylthymine, 1-propyl-5-ethyluracil, 9-propyladenine has been described previously [12,16]. TP-U, TP-ETU and TP-A were synthesized by the procedure analogous to that described for TP-T [16]. The details of the isolation and purification procedure, and selected spectral data for the dyads are presented in Supplementary data. The site of the attachment of the trimethylene spacer to the N-9 position of the 6-thiopurine ring was unequivocally proven by the observation of the long-range ^1H–^{13}C coupling between the 6-thiopurine α-methylene proton and the purine ring C4 atom in the ^1H–^{13}C HMBC spectrum.

2.2. Methods

2.2.1. Instrumentation

The UV absorption spectra were recorded with a Cary 300 Bio (Varian) spectrophotometer. The NMR spectra were measured at 400 MHz (^1H) with Bruker AVANCE II or at 600 MHz (^1H) with Bruker AVANCE spectrometers. The chemical shifts (δ) are in ppm relative to TMS. High-resolution MS spectra (MALDI) were measured on Q-Tof spectrometer. HPLC chromatography was performed on a Waters 600 E instrument equipped with a Waters 991 Photodiode Array UV–VIS detector (PDD) using a reversed phase column: Waters X-Terra RP18 (5 μm; 4.6 mm × 150 mm) for analytical runs and Waters X-Terra RP18 (7 μm; 7.8 mm × 150 mm) for preparative separations. A gradient of CH_3CN in H_2O was used as an eluent. Water was doubly distilled and purified using Simplicity Ultrapure Water System (Millipore).

2.2.2. Nanosecond laser flash photolysis

The nanosecond laser flash photolysis setup and its data acquisition system have been described in detail [17]. All experiments were carried out in a rectangular quartz optical cell (1 cm × 1 cm) using argon-saturated solutions in a phosphate buffer (0.01 M, pH 5.8). The samples were excited with 1–5 mJ laser pulses at λ = 355 nm.

2.2.3. Analytical scale irradiation and quantum yield determination

Solutions (2.6 mL) of TP-U, TP-T, TP-ETU and TP-A ($c \sim 1 \times 10^{-4}$ M) in phosphate buffer were placed in a 1 cm × 1 cm UV cell and deoxygenated by bubbling with argon. Irradiations were performed at λ = 351 nm with an argon ion laser (Innova) equipped with a home-built selector based on a double Pellin–Brocka prism. For quantum yield determinations, the samples were irradiated with the λ = 313 nm line, isolated from a high-pressure mercury lamp. Irradiation was continued to a low substrate conversion, and concentration changes were determined by HPLC analyses. Uranyl oxalate actinometry was used [18].

2.2.4. Preparative irradiations

The compound TP-T (15 mg) was dissolved in water (~500 mL), and the solution was irradiated in portions (80 mL) with a 150-W high pressure immersion mercury lamp through a pyrex filter under an argon atmosphere. Irradiation was continued to ca. 60% conversion of the substrate. The irradiated solutions were collected and concentrated under reduced pressure. An analogous procedure was used in preparative irradiation of TP-ETU. The compound HP-CH_2-U, the single product formed from TP-T, was isolated by preparative HPLC using 5% aq. CH_3CN in the isocratic mode (10 min), followed by gradient (5% aq. $CH_3CN \rightarrow$ (15 min)

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