A Minute Amount of S-Puckered Sugars Is Sufficient for (6-4) Photoproduct Formation at the Dinucleotide Level

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Supporting Information

ABSTRACT: The di-2′-α-fluoro analogue of thymidylyl(3′,5′)-thymidine, synthesized to probe the effect of a minimum amount of S conformer on the photoreactivity of dinucleotides, is endowed with only 3% and 8% of S sugar conformation at its 5′- and 3′-end, respectively. This analogue gives rise to the (6-4) photoproduct as efficiently as the dithymine dinucleotide (74% and 66% at the 5′- and 3′-end, respectively) under 254 nm. Our results suggest that the 5′-N, 3′-S conformer gives rise to the (6-4) photoproduct.

Exposure of DNA to UV light results mostly in chemical modifications at adjacent pyrimidine sites. In cells, such modifications can be repaired or lead to senescence, death, or mutation. Therefore, DNA photoproducts play a considerable role in human health through their involvement in skin cancer. Cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts ((6-4) PPs) represent the two major classes of DNA photoproducts (Scheme 1). Although CPDs have been found to be the most deleterious PPs in repair-proficient cells, (6-4) PPs would be considerably more dangerous in repair-capacity-saturated cells. Therefore, identification of the processes leading to (6-4) PP, including DNA conformational parameters, is of utmost importance. The dithymine dinucleotide TpT (1) is a good model to study DNA photoreactivity. As such, we currently dissect its photoreactivity as well as that of conformationally restricted analogues.

Sugar conformation is one of the key factors governing global conformation of nucleic acids even at the dinucleotide level. In aqueous solution, the sugar puckers of nucleos(t)ides exists as an equilibrium mixture whose extreme forms are the North (N) and South (S) conformers. Substitution at the nucleos(t)ide 2′-position dramatically influences this equilibrium. Photochemical investigation of T1pT1, the locked TpT analogue, has evidenced that exclusive N conformation at each dinucleotide end precludes (6-4) PP formation, CPD being the sole PP formed. Therefore, S-type sugar conformation to promote relevant intramolecular thymine moieties overlap geometry for (6-4) PP to form. Therefore, we decided to determine the minimum amount of S conformer necessary to get (6-4) PPs. To study this hypothesis, we designed a modified dithymine dinucleotide not locked as T1pT1 but whose expected N conformer population would nevertheless approach 100% as closely as possible at each end. 2′-α-Fluorosubstitution of pyrimidine nucleosides is known to drive the N population of the sugar conformation over 85%. Therefore, we synthesized the dinucleotide analogue of TpT containing a fluorine atom at the 2′-α-position of each sugar residue (2′-α-fluorothymidylyl-(3′,5′)-2′-α-fluorothymidine, TαFpTαF2) to study its conformation and photoreactivity.

Dinucleotide 2 was prepared from 2′-α-fluorothymidine following the procedure of Ikeda et al. and Williams et al. (Scheme 2). In brief, dimethoxytritylation of 2′-α-fluorothymidine provided 8 in quantitative yield. 3′-Acetyl-2′-α-fluorothymidine 9 was prepared through a one-pot acetylation/ detritylation procedure from 8 in 60% yield. Modified nucleoside 8 also afforded known phosphoramidite 10, which was then condensed with alcohol 9. Condensation was performed in acetonitrile and in the presence of 5-ethyliothetrazole as activating reagent, instead of the more common 1H-tetrazole, due to the known lower reactivity of 2′-fluorophosphoramidites compared to their unsubstituted counterparts. The corresponding phosphite triester intermediate was subsequently oxidized in the presence of I2 to give intermediate 11 in 60% yield. Deprotection of 11, performed using concentrated aqueous NH4OH and then 80% aqueous acetic acid, afforded 2 in 73% yield.

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Note
Delightfully, the NMR-based PSEUROT+JHF analysis of 2 indicated that, in solution, both sugar residues are highly predominantly, but not exclusively, in the N conformational domain. North conformer population was calculated to be 97% and 92% at the 5'- (PN = 22.8°) and 3'- (PN = 22.5°) end, respectively (Supporting Information). Exposure of an aqueous solution of 2 to 254 nm light afforded the c,s CPD (3) and the (6-4) PP (4) (Supporting Information). The mass spectra of 3 and 4 displayed a quasi-molecular ion peak at m/z 581 (\((M - H)^-\)). The presence of two proton singlets at \(\delta 7.88\) and \(\delta 5.12\) and two methyl protons at \(\delta 2.30\) and \(\delta 1.79\) in the \(^1H\) NMR spectrum of 4 was the signature of a (6-4) adduct while the presence in the \(^1H\)
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Figure 2. CD difference spectra at 20 °C of 1 and of 2 normalized with respect to the positive band amplitude of 1.

NMR spectrum of 3 of two shielded methyl signals (δ 1.52 and 1.43) attested the presence of a CPD. UV absorption of 4 at λ_{max} = 328 nm and lack of a UV absorption maximum above 240 nm for 3 further confirmed the assigned structures. The cis–syn (c,s) stereochemistry of the cyclobutane 3 was deduced from nOEs between H6 T_{αfP} and H3′ T_{αfP}, H6 T_{αfP} and CH3 T_{αfP}; H6 T_{αfP} and CH3 H2′ pT_{αf}; H6 H2′ pT_{αf}; H6’ pT_{αf}; and H6’ pT_{αf} and CH3’ pT_{αf}. For the (6-4) PP 4, observed nOEs established the C5 (R) and C6 (S) stereochemistry (H6 T_{αfP} and H3′ T_{αfP}; H6 T_{αfP} and H2′ T_{αfP}; H6 T_{αfP} and CH3 T_{αfP}; H6 T_{αfP} and CH3; pT_{αf}; H6’ pT_{αf}; and CH3’ pT_{αf}; H6’ pT_{αf}; and H2′ pT_{αf}; H6’ pT_{αf} and H3’ pT_{αf}). Thus, 3 and 4 arise from cycloaddition reactions between the two thymine bases of 2 in an anti glycosidic bond conformation as in the case of 1.

Photochemical reaction kinetics and quantum yield (Φ) of PP formation, from 2 with respect to 1, were determined (Supporting Information) as previously reported. Kinetic studies revealed a similar initial rate of formation of (6-4) PPs 4 and 6 obtained from UV exposure of 2 and 1, respectively (Figure 1). The higher rate of disappearance of 2, with respect to 1, was nicely correlated with a higher initial rate of formation of CPD 3 compared to that of TpT-derived CPD 5 (Figure 1). While Φ of CPD formation from 2 ((2.7 ± 0.3) × 10^{-2}) was estimated to be ca. twice higher than that from 1 ((1.1 ± 0.05) × 10^{-2}), Φ of (6-4) PP formation from 2 ((0.13 ± 0.02) × 10^{-2}) was surprisingly found to be quite similar to that of TpT 1 ((0.10 ± 0.05) × 10^{-2}), whose S conformer population is 74% and 66% at its 5′- and 3′-end, respectively.

Conformers in solution are in an equilibrium mixture of intramolecular stacked and unstacked species, among which only intramolecular stacked species can yield photoreaction products. Thus, we investigated the stacking properties of 2 by circular dichroism (Supporting Information). The CD difference spectrum of 2 and 1 is presented Figure 2.

The similar shape of the CD difference spectrum of 2 and 1 indicated that the average intramolecular base stacked population adopted by 2 and 1 is similar in terms of geometry (Figure 2). However, the ca. 3-fold increase in molecular ellipticity at λ_{max} 278 nm supported the idea that T_{αfP}T_{αf} (2) is 3 times more stacked than TpT. Therefore, the increased formation efficiency of CPD 3, compared to 5, may be correlated with the elevated degree of stacking, associated with a favorable geometry, of its precursor (2), compared to TpT. This hypothesis is in line with the involvement of the initial ground-state geometry of the two reacting thymine residues in CPD formation. In contrast, since Φ of (6-4) PP from 2 is similar to that from 1 and since 2 is 3 times more stacked than 1, formation of (6-4) PP 4 is not linearly correlated with the stacking level of 2. If the population of the S conformer is the rate-limiting step in (6-4) PP formation, this could indicate that (6-4) PP arises either from non-CD detectable ground state conformations of minor favorably stacked species or from species whose geometry is different from the one of the ground state. If the population of the S conformer is not the rate-limiting step in (6-4) PP formation, this study supports the idea that other factors such as excited state geometries/properties govern the formation of (6-4) PP.

In conclusion, whereas T1pT1 is incompetent to afford (6-4) PP, TpTpT_{αf} 2, whose S sugar conformation is 3% and 8% at the 5′- and 3′-end, respectively, can provide (6-4) PP as efficiently as TpT whose S conformer is 74% and 66% at the 5′- and 3′-end, respectively. For 2, such sugar conformational population represents dinucleotide-population conformer probabilities of NN (89.9%) population in equilibrium with 7.6, 2.3, and 0.2% of NS, SN, and SS species, respectively. Considered alone, the amount of S conformers cannot be used to anticipate the capacity of a dinucleotide to form (6-4) PP. However, our results clearly indicate that, at the dinucleotide level, conformational changes induced by the presence of S-puckered sugars in amounts as minute as 3% and 8% at the 5′- and 3′-end, respectively, are sufficient to allow (6-4) PP formation. Since an S sugar conformation at the 5′-end of a dinucleotide is known to be detrimental to intramolecular stacking and that only stacked species can afford PP, this strongly supports the idea that an S conformer at the 3′-end is essential for (6-4) PP formation. The (6-4) photoproduct would, therefore, result from NS dinucleotide conformers.

### EXPERIMENTAL SECTION

**General Remarks.** Solvents and chemicals used for the reactions were purchased from commercial suppliers. Dichloromethane, acetonitrile, N,N-diisopropylethylamine (DIEA), ethyl acetate (AcOEt), and heptane were dried by distillation from calcium hydride. Pyridine and triethylamine (TEA) were dried by distillation from KOH and kept over KOH. TLC was performed on silica gel plates (Kieselgel 60, F254) with...
detected by UV and visualization by spraying with a methanolic solution of sulfuric acid. Chromatography was performed on silica gel 60, particle size 35–70 μm, unless otherwise stated. Medium pressure reverse-phase chromatography was performed on a LiChroPrep RP 18.

1H NMR and 13C NMR spectra were recorded on 300 or 500 or 600 MHz spectrometers. Observed chemical shift (δ) values are given in ppm and coupling constants (J) in Hz. The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), T reap and T rap represent the S'-end and the S''-end nucleoside residues, respectively, of 2−4. 1H NMR and 13C NMR chemical shifts were calibrated using residual solvent signals at the following values: CD3OD δH 3.31 and δC 49.15; D2O δH 4.80. For CDCl3 and DMSO-d6, δ values were reported from internal TMS (δ 0.00). 1H NMR spectra recorded in D2O were calibrated from dioxane (δ 6.78 ppm).

3−O-Acetyl-2′−α-fluorothymidine (9). To a solution of 3′−O-dimethoxytrityl-2′−α-fluorothymidine 8 (0.216 g, 0.38 mmol) in dry pyridine (5.3 mL) was added Ac2O (1.43 mL, 1.52 mmol). The mixture was stirred for 24 h, then evaporated to give residue, and the residue was purified by flash silica gel chromatographies (30−100% AcOEt in heptane and 0−10% CH3OH in CH2Cl2) to give 9 (0.070 g, 60% as a white foam. 1H NMR (300 MHz, CDCl3); δ 7.53 (1H, s), 5.93 (1H, dd, J = 3.4, 16.8 Hz), 5.25 (1H, ddd, J = 3.4, 5.0, 29.8 Hz), 5.25 (1H, dd, J = 5.0, 6.2, 12.2 Hz), 4.18 (1H, m), 3.89 (1H, m), 2.10 (1H, m), 1.70 (1H, d, J = 2.4, 12.5 Hz), 2.11 (3H, s), 1.85 (3H, s). 13C NMR (62.5 MHz, CDCl3); δ 170.4, 161.4, 150.5, 137.5, 111.7, 90.8 (d, J = 193.1 Hz), 90.7 (d, J = 33.7 Hz), 82.0 (d, J = 14.8 Hz), 60.9, 20.7, 12.5. 19F NMR (282 MHz, CDCl3); δ −201.86. HRMS (ESI+ mode) (M + Na)+: calcd. for C14H15NO4F2PNa+ 325.0812, found 325.0813.

P-Cyanoethyl-S′−O-dimethoxytrityl-2′−α-fluorothymidylyl-(3′,5′)-3′-O-acetyl-2′−α-fluorothymidine (11). Compounds 9, 10,1,4,5 and 5′-ethyldithiotetrazole were dried over P2O5 under vacuum. The mixture was stirred for 24 h, then evaporated to give residue, and the residue was dried under vacuum, then treated with Ac2O (2 × 20 mL). The organic layer was dried over anhydrous Na2SO4, and evaporated to give crude P-Cyanoethyl-S′−O-dimethoxytrityl-2′−α-fluorothymidine. The crude product was taken in 80% aqueous AcOH (19 mL) and stirred for 4 h at room temperature. The mixture was evaporated and the residual oil was purified by two successive flash silica gel chromatographies (30−100% AcOEt in heptane and 0−10% CH3OH in CH2Cl2) to give 10 (0.293 g, 0.38 mmol) in anhydrous CH2CN under argon at room temperature, 9 (0.097 g, 0.32 mmol) and 5′-ethyldithiotetrazole (0.138 g, 1.06 mmol) were added. The reaction was stirred for 30 min. A 0.2 M iodine solution (0.145 g, 0.57 mmol) in THF/H2O (2:1, v/v) was added to the reaction, and the mixture was stirred for 25 min. A saturated sodium thiosulfate solution was added until the solution became colorless. The mixture was diluted with CH2Cl2 and washed with water. The organic layer was dried over anhydrous Na2SO4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (50−100% AcOEt in heptane) to give 11 (0.185 g, 60% as a white foam. 19F NMR (282 MHz, CDCl3); δ −195.98, −197.48, −201.54, −202.61. 13C NMR (121 MHz, CDCl3); δ −2.35, −2.68. HRMS (ESI+ mode) (M + Na)+: calcd. for C20H24N4O12F2P 581.1096, found 581.1091.
REFERENCES


Note

The authors declare no competing financial interest. For convenience, the name 2′-α-fluoro-2′-fluoromethyluridine is preferred instead of 2′-deoxy-2′-fluoro-3-methyluridine.

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