Photochemical Reactions of Microcrystalline Thymidine

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Supporting Information

ABSTRACT: Nucleoside/nucleotide/oligonucleotide photoreactions usually result in a number of products simultaneously due to a wide range of conformers existing at a given time. Such a complicated reaction pattern makes it difficult for one to focus on a single DNA photoproduct and elucidate the requirements for its formation. A rare example of thymidine photoreaction in microcrystals is reported, where 5-thyminyl-5,6-dihydrothymine, e.g., the spore photoprotein (SP), is produced as the dominant species in ~85% yield. This unprecedented high yield clears the major obstacle for future SP photochemistry studies in detail.

DNA conformations are known to control the outcome of DNA photoreactions.1−4 In a normal cell, the millions of nucleobases in the genome may adopt an equally large number of stacking conformations at a given time owing to the constant thermal motion. As a consequence, although cyclobutane pyrimidine dimers (CPDs) are usually the most abundant DNA photoproducts in a UV irradiated cell, their production is always accompanied by many other species. The lack of “clean” DNA photoproduct formation drastically hinders the understanding of the general DNA photochemistry.

To address this problem, unnatural nucleotides were adopted in in vitro DNA photochemical studies. For instance, using a dinucleotide TpT composed of locked nucleic acids in which the furanose moieties are locked at the C3′-endo conformation, an enhanced stacking interaction between the two thymine rings was achieved.5 Such a favorable interaction eliminates the minor population of TpT conformers leading to the formation of pyrimidine (6−4) pyrimidine photopродuct (6−4PP),6 the other common pyrimidine phototolysis, resulting in CPD as the exclusive photoprotein upon UV irradiation.

Bacterial endospores represent an in vivo system where a certain type of DNA photoreaction is favored. Endospores are only ~30% hydrated comparing with normal vegetative cells; such a low hydration level changes the genomic DNA to an A-conformation. The A-DNA is solidified by a group of DNA binding proteins named the small acid soluble proteins, e.g., the spore photoprotein (SP).7,8 A recent molecular simulation using the structure of a nucleoprotein formed between a small acid soluble protein and 10-mer oligo(dG)−oligo(dC) found that after replacing the sixth and seventh GC with AT pairs, the Cs of one T was only 3.4 Å away from the −CH3 of another T.7,10 This distance is shorter than the 3.9 Å between the two Cs positions, which is required to connect in CPD formation.7 The corresponding moieties involved in the 6−4PP formation are even further. These results offer a rationale on how the DNA conformation promotes SP photochemistry and quenches other photoreactions.

SP can be formed in vitro via solid state (ice or dry film) DNA photoreactions; the numerous conformations adopted by thymine residues, however, determine that formation of SP is always accompanied by CPD and 6−4PP.11−14 UV irradiation of thymidine at solid state produces dinucleoside SP.13 Diastereoisomers are formed under the same H atom abstraction mechanism.16 As proved by Ames et al., these SP diastereoisomers are formed under the same H atom abstraction followed by radical recombination mechanism.16

Via slow evaporation of the thymidine methanol solution, Douki et al. obtained a thymidine thin film. UV irradiation of this film afforded the 5R- and 5S-SP in ~1:20 ratio; few other thymidine photoproducts were produced. This result suggests that thymidine residues in the dry film stack into conformations favoring SS-SP formation. However, the low yield (1%) indicates that most of the thymidine residues in the dry film are nonreactive and release the excitation energy via thermal decay. This could be owing to the fact that only layers at the film surface are exposed to UV light while the bulk of the material is protected from reaction, although the possibility that the majority of thymidine residues adopt nonreactive conformations cannot be excluded. Crystals represent a special solid state, where molecules adopt a homogeneous structure within the whole...
lattice. The high stereoselectivity and specificity of photoreactions in crystals are known for many years.18,19 We thus wonder whether thymidine microcrystals can mimic the DNA environment in spores, leading to a clean SP formation under UV irradiation.

Following a literature protocol, we first prepared thymidine single crystals via slow evaporation of a thymidine aqueous solution.20 Irradiation of the single crystals under unfiltered UV light centered at 254 nm for 1 h indeed results in one product at ≤0.1% yield; the only other species isolated via HPLC is the unreacted thymidine. This reaction thus represents a very rare example of "clean" nucleoside photoreaction. By comparing with the SP standards prepared by organic synthesis,16 the product is confirmed as the 5S-SP. The low SP yield, however, indicates that the microcrystalline photoreaction may still suffer from the possible limitation of the dry film photoreaction that only the thymidine molecules near the surface react. To improve the reaction yield, we grinded the crystals into fine powder before UV light was applied. Even under a constant agitation, the yield of 5S-SP was still ~1.0% after 24 h under 254 nm UV light (Figure 1A); a prolonged photoreaction led to little improvement.

To improve the yield of the crystalline photoreaction, Veerman et al. suspended the microcrystals of dicumyl ketone in water and irradiated the suspension under constant stirring.21 A nearly stoichiometric conversion of reactant to product was obtained after 24 h of UV irradiation.21 We therefore decided to adopt a similar strategy for our thymidine photoreaction. As thymidine is soluble in water, but not in many organic solvents, we mixed 2 mg of thymidine microcrystal powder with 2 mL of hexane and irradiated the suspension under 254 nm UV light with vigorous stirring in a cylinder quartz UV cuvette. To our delight, the yield of 5S-SP was improved drastically to 9.3% after a 2.5 h reaction.22

To reveal whether the solvents used alter the microcrystalline state and subsequently change the photoreaction yield, we surveyed a number of organic solvents, including diethyl ether, methyl t-butyl ether (MTBE), dicholomethane, dichloroethane, ethyl acetate, acetone, methanol, ethanol, etc., for the SP photoreaction.22 MTBE was found to be the best solvent, resulting in 5S-SP at 30% yield after a 2.5 h reaction under 254 nm UV irradiation. We therefore chose MTBE for further thymidine photoreactivity studies.

Irradiation of 2 mg of thymidine microcrystals suspended in 2 mL of MTBE results in a linear formation of 5S-SP in the first several hours of the reaction (Figure 2). The reaction under unfiltered UV light peaked at 302 nm is ∼6-fold slower than that under the 254 nm UV light. Both reactions were very clean in the first 12 h; little other products were observed in the HPLC chromatograph (Figure 1B). The reaction under 254 nm UV light plateaued in 12 h with the yield of SP reaching 68 ± 3%. After 24 h, the yield decreased to ∼63% with subsequent formation of a number of new peaks in the HPLC chromatograph. We tentatively ascribe this observation to irradiation-induced SP decomposition processes.
reaction under 302 nm UV light was slower, the overall yield of SP is higher. An 84 ± 2% yield was obtained after a 32 h reaction. It is worth pointing out that both yields represent a drastic improvement comparing with SP formation in other in vitro systems, where the maximum yield observed was ~1%.16,23

The clean SP formation likely results from the homogeneous stacking conformation in the thymidine crystal, which quenches other side reactions. To reveal the thymidine conformation supporting SS-SP formation, we reanalyzed the thymidine structure solved by Young et al.20 The analysis reveals that the thymidine residues stack on each other to form different layers (Figure 3A). These layers are not perpendicular to the vector defined by the centers of stacked thymine rings; instead they exhibit a dihedral angle of ~62°. Such a packing mode places the methyl group of one thymine (a, Figure 3B) right above the other thymine ring (b, Figure 3B); the shortest distance between an H atom in −CH₃ and the C6 of another thymine is ~3.2 Å (blue arrow), which is close to the 3.4 Å found in the molecular simulation and supports the key H-abstraction step to initiate SP formation.16,23

Figure 3. (A) Molecular packing in the thymidine single crystal. The distance between two thymidine rings is ~3.1 Å, close to the 3.36 Å average rise found in the SASP-oligo(dG)-oligo(dC) nucleoprotein.7 (B) The shortest distance between an H atom in −CH₃ and the C6 of another thymine is ~3.2 Å (blue arrow), which is close to the 3.4 Å found in the molecular simulation and supports the key H-abstraction step to initiate SP formation.16,23

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thymidine single crystal structure to explain the observed SP formation; analysis of the unique "S" shape reaction curve allows us to conclude that subtle conformational changes may occur to facilitate SP photochemistry. The ~85% yield in 302 nm reaction is unprecedented in DNA photochemical studies. This clean and high-yield SP formation opens the door for future SP photochemistry elucidation in detail. Further SP mechanistic investigations using thymidine microcrystals are currently underway.

**ASSOCIATED CONTENT**

Supporting Information

Synthesis and characterization of SS-SP product. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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