One-electron oxidation of a pyrenyl photosensitizer covalently attached to DNA and competition between its further oxidation and DNA hole injection

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Abstract

The photosensitized hole injection and guanine base damage phenomena have been investigated in the DNA sequence, 5′-d(CATGPyCG2TCCTAC) with a site-specifically positioned pyrene-like (Py) benzo[a]pyrene 7,8-diol 9,10-epoxide – derived N2-guanine adduct (GPy). Generation of the Py radical cation and subsequent hole injection into the DNA strand by a 355 nm nanosecond laser pulses (~4 mJ/cm2) results in the transformation of GPy to the imidazolone derivative IzPy and a novel GPy* photoproduct that has a mass larger by 16 Da (M+16) than the mass (M) of GPy. In addition, hole transfer and the irreversible oxidation of G2, followed by the formation of Iz2 was observed (Yun et al., J. Am. Chem. Soc. 2007, 129, 9321). Oxygen-18 and Deuterium isotope labeling methods, in combination with an extensive analysis of the MS/MS fragmentation patterns of the individual dGPy* nucleoside adduct and other data, show that dGPy* has an unusual structure with a ruptured cyclohexenyl ring with a carbonyl group at the rupture site and intact guanine and pyrenyl residues. The formation of this product competes with hole injection and thus diminishes the efficiency of oxidation of guanines within the oligonucleotide strand by at least 15% in comparison with that in the dGPy nucleoside adduct.

Introduction

Benzo[a]pyrene, an extensively studied pre-carcinogenic polycyclic aromatic hydrocarbon (1), is metabolized by cytochrome P450 enzymes to highly reactive diol epoxides, including the biologically significant intermediate (+)-7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (anti-BPDE) (2). This diol epoxide forms covalent adducts with DNA by binding predominantly to the exocyclic amino group of guanine via its C-10 position. The addition of the N2-guanine to the C-10 position of BPDE can occur either by cis or trans mechanisms, thus forming two stereoisomeric adducts (3). The (+)-trans-addition product is the major adduct found in cellular DNA (4), and is designated as 10S (+)-trans-[Py]-N2-dG, or dGPy (Figure 1). We have been studying the structural and functional characteristics of such lesions site-specifically inserted into oligonucleotides of defined sequence context for some time (5-8). The (+)-trans and stereochemically similar adducts have pyrene (Py)-like spectroscopic properties (9) with a strong absorption maximum in the 300 – 350 nm region and a fluorescence emission in the 390 – 440 nm region. The fluorescence of this product is strongly quenched by a photoinduced electron transfer mechanism (10-12). The nature of the electron transfer mechanism was investigated in detail.
by laser flash photolysis methods that showed that the photoexcited Py residue can oxidize
the adjacent guanine residue; the ion radical pair thus formed recombines non-radiatively,
thus accounting for the observed fluorescence quenching (12-14). The spectroscopic
properties of the GPy adduct embedded in DNA have been used extensively to study the
conformational properties of these lesions (15-17).

More recently we have used the GPy adduct site-specifically incorporated into the
oligonucleotide 5’-d(CATG1PyCG2TCCTAC) as a photosensitizer to inject holes into this
sequence and to monitor oxidative damage at the Py-modified base, dG1Py, and the single,
more distant unmodified guanine residue G2 (18). The Py residue was selectively
photoexcited with 355 nm nanosecond laser pulses (~4 mJ/cm2) that generated the Py•+
radical cation by a two-photon induced ionization mechanism, as well as hydrated electrons
that were trapped by molecular oxygen to form superoxide anions, O2•− (18).

Transient absorption spectroscopy methods were utilized to ascertain that Py•+ selectively
oxidized the guanine residue to which it is attached (19); this is equivalent to ‘hole injection’
into the oligonucleotide (18) that results in the formation of the Py-modified guanine radical
cation, (G1Py)+ (18, 19). These radical cations rapidly deprotonate to form the neutral
guanine radicals bearing Py residues, G1Py(−H)+ radicals. The latter decay by at least two
competitive mechanisms: (1) an electron transfer mechanism that generates the G2(−H)+
radical that becomes irreversibly oxidized (18), or (2) transformation of G1Py(−H)+ to
various oxidized products that included 2,5-diamo-4H-imidazolone lesions bearing
pyrenyl residues (I2Py), generated by the combination of G1Py(−H)+ radicals with
superoxide anion radicals (Figure 1). A previously unknown lesion, G1Py*, with a mass (M +16),
which is by 16 Da greater than the mass (M) of the parent G1Py, was also detected in
the irradiated oligonucleotide d(CATG1PyCG2TCCTAC). These competitive reaction
pathways are summarized in Figure 1. The formation of this product competes with hole
injection and thus diminishes the efficiency of oxidation of guanines within the
oligonucleotide strand.

The mass M + 16 is frequently associated with the formation of 8-oxo-7,8-dihydro-2’-
deoxyguanosine (8-oxodG) lesions, a common oxidation product of guanine (20). In order to
investigate this possibility, the M+16 oxidation product dG1Py* was excised from the
oligonucleotide by enzymatic digestion methods, and the properties of the dG1Py*
nucleoside were compared to those of the authentic anti-[Py]-N2-8-oxodG adduct standard,
8-oxodGPy (18). However, the chromatographic properties of the M+16 product dG1Py*
were different from those of the 8-oxodGPy standard, thus ruling out this possibility and the
nature of this product was not further investigated.

In this work we investigated the structure of the dG1Py* product in detail by mass
spectrometric MS/MS and spectroscopic methods. Our MS/MS studies were facilitated by
generating larger quantities of the dG1Py* nucleoside standard than was possible by
enzymatically digesting the photoirradiated oligonucleotide 5’-
d(CATG1PyCG2TCCTAC). The structure of this M+16 dG1Py* nucleoside standard was
established by LC-MS/MS methods using 18O and deuterium isotope labeling methods and
analysis of the fragmentation patterns. We conclude that the M+16 nucleoside product
represents an oxidized form of the photosensitizing dG1Py moiety that has an unusual
structure with a ruptured cyclohexenyl ring, that leaves the guanine and pyrenyl residues
remain intact. The formation of this product competes with hole injection and thus
diminishes the efficiency of oxidation of guanines within the oligonucleotide strand.
Experimental

Materials

All chemicals (analytical grade) were used as received. The 10S (+)-trans-anti-[Py]-N²-dG adduct dGP² was synthesized by a direct reaction of dG with racemic anti-BPDE obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository. The dGP² products were isolated and purified by reversed-phase HPLC methods as previously described (3). The structure of the adduct was confirmed by LC-MS/MS methods and circular dichroism analysis.

Photochemical synthesis of the M+16 dGP² nucleoside adduct

A 25 nmol sample of the 10S (+)-trans-anti-[Py]-N²-dG nucleoside in 0.25 mL air-equilibrated phosphate buffer solutions (pH 7) was photolysed by a train of 355 nm nanosecond Nd:Yag laser pulses (4 mJ/cm², 10 Hz) for 20 s. This procedure was repeated with fresh samples of the nucleoside in order to accumulate sufficient amounts for detailed analysis. The end products thus obtained were separated by reversed-phase HPLC methods. Typical elution conditions included a 0 – 15% linear gradient of solvent A (1:1 mixture of solvent B and water) in solvent B (3:1 mixture of methanol and acetonitrile) for 60 min, was employed a flow rate of 1 mL/min. The products were detected by their absorbance at 260 nm. Under these conditions the M+16 nucleoside eluted after 10.6 min, and the unmodified nucleoside G² eluted at 14.5 min. The HPLC fractions containing the M+16 adduct were evaporated under vacuum to remove organic solvent and were purified by a second HPLC cycle. The purified M+16 adducts were desalted by reversed-phase HPLC using the following mobile phases: 5 mM ammonium acetate (10 min), deionized water (10 min), and an isotropic 50 : 50 methanol and H₂O mixture (15 min), and then subjected to LC-MS/MS analysis.

LC-MS Assay

The end products of the 10S (+)-trans-anti-[Py]-N²-dG photolysis were identified using an Agilent 1100 Series capillary LC/MSD Ion Trap XCT and an Agilent 1100 Series LC/MSD VL mono quadrupole mass spectrometer equipped with an electrospray ion source. In typical ion trap experiments 1 – 8 μL of the sample solutions were injected in a narrow bore Zorbax SB-C8 column (50 × 2.1 mm i. d.) and eluted with an isocratic mixture of methanol and water (55 : 45) with 0.1% formic acid as the mobile phase, at a flow rate of 0.25 mL/min. In mono quadrupole experiments, 10 – 30 μL of the sample solutions were injected into a Zorbax SB-C8 column (150 × 4.6 mm i. d.) and eluted with an isocratic mixture of methanol and water (55 : 45) with 0.1% formic acid as the mobile phase at a flow rate of 0.5 mL/min.

Results and Discussion

The 5’-d(CATG¹PyCG₂TCCTAC) oligonucleotide was dissolved in air-equilibrated phosphate buffer solutions (pH 7.0) and irradiated with 355 nm nanosecond laser pulses (~4 mJ/cm²). The photoproducts were isolated by reversed-phase HPLC and identified by LC-MS methods. In HPLC separations of the products of photolysis, the major end product with a mass of M+16, eluted at 22 min which is greater by 16 Da than the mass of the parent adduct (M), which elutes at 28 min (typical results are shown in (18)). The product with mass (M+9) eluted at 24 min was formed in smaller quantities and was identified as the imidazolone dL²⁷ product bearing an intact Py residue. The dG¹Py* nucleoside product was isolated by enzymatic digestion of the irradiated oligonucleotide d(CATG¹PyCG₂TCCTAC). Its MS/MS properties were identical to those of an M+16 dGP² nucleoside product obtained by the photolysis, using identical irradiation conditions, of the
10S (+)-trans-anti-[Py]-N^2-dG nucleoside adduct, dG^Py (data not shown). The photolysis of the dG^Py nucleoside also generated the nucleoside dIz^Py. Thus the same products are formed within the oligonucleotide as at the nucleoside level, and we conclude that dG^1Py* is the same as the M+16 dG^Py* nucleoside standard. In addition to the Iz and M+16 oxidation products, laser pulse excitation induces the detachment of the pyrenyl residues. The formation of unmodified oligonucleotides with intact G replacing the adduct G^Py was clearly observed by gel electrophoresis methods (18, 21). The unmodified oligonucleotides were isolated by reversed-phase HPLC and identified by MALDI-TOF/MS methods (data note shown). In our typical experiments, the yields of the unmodified sequences were in the range of 0.5 – 0.6 %. The laser pulse – induced detachment of Py with similar yields was also observed in the case of the dG^Py nucleoside adducts.

Irradiation of either the nucleoside dG^Py or the oligonucleotide 5'-d(CAT[G1Py]CG2TCCTAC) under the same conditions of laser energy and irradiation time generates the same amounts of the M+16 products with yields of 8%, as shown in Figure 2. The recovery of the unreacted dG^Py (67±7 %) is somewhat greater than that of the unmodified oligonucleotide 5'-d(CAT[G1Py]CG2TCCTAC) (57±6 %); this relatively small difference was not further investigated.

The formation of M+16 product at dG^1Py competes with the formation of the dG^1Py(−H)* radical, which is equivalent to hole injection into the oligonucleotide. The dG^1Py(−H)* radical decays by competitive pathways to dIz^Py and an electron transfer step from dG^1Py(−H)* to G2 that can result in the latter’s irreversible oxidation. These competitive pathways are summarized in Figure 1. From Figure 2, it is evident that the oxidation of the Py photosensitizing moiety that results in the formation of the M+16 oxidation product dG^1Py* diminishes the yield of hole injection by ~ 15%. These results demonstrate that the oxidation of the photosensitizer can limit the efficiency of hole injection, and that a complete understanding of photoinduced hole injection and electron transfer phenomena in DNA should include information about the fates of the photosensitizing moieties and their chemical and photochemical stabilities. In our case, the dG^1Py* retains its photosensitizing properties that can lead to the near complete degradation of oligonucleotides (10, 18, 21). In the following, we establish the nature of the primary photoproduct M+16 dG^Py* product and propose a mechanistic pathway of its formation.

The ^18O and D isotope labeling of the dG^Py* nucleoside adducts

The difference in the masses of dG^Py* and dG^Py, is most likely associated with the insertion of an oxygen atom (16 Da) into the parent adduct. In oxidative processes, the sources of O atoms are typically either molecular oxygen or water molecules. Typically, the insertion of O-atoms into organic molecules (RH) to form ROH products, results in the appearance of one solvent-exchangeable proton. An example is the hydroxylation of the pyrenyl ring system induced by the photolysis of pyrene in oxygenated aqueous solutions (22). In this reaction, the insertion of each O-atom in the aromatic ring to form OH group results in the appearance of an additional exchangeable proton. In our experiments, we tracked the insertion of O-atoms into dG^Py using ^18O and deuterium isotope labeling experiments.

We synthesized the dG^Py* nucleoside in H2^18O solutions equilibrated with air containing normal molecular oxygen, ^16O2. The molecular ion, [M+H]^+ of the adduct is observed at m/z 588.2 (Figure 3A) that is by 2 Da greater than the control dG^Py* product (mass M+16) generated in the same manner in H2^16O solution (Figure 3B). This mass difference of 18 Da relative to the parent dG^Py adduct at m/z 570.2 (Figure 3E), provides straightforward evidence that the oxygen atom originates from H2^18O and not from ^16O2.
The numbers of exchangeable protons in the dG\textsuperscript{Py\*} oxidation product were determined from the mass spectra after proton exchange in D\textsubscript{2}O (99.9\%) by repeated lyophilization, followed by redissolving the products in D\textsubscript{2}O (Figures 3C and 3D). Using D\textsubscript{2}O/acetonitrile mixtures as the mobile phase in LC-MS analysis, we found that the molecular ions of dG\textsuperscript{Py\*} and dG\textsuperscript{Py} are observed at \textit{m/z} 594.2 (Figure 3C) and \textit{m/z} 578.2, respectively (Figure 3D). In contrast, LC-MS analysis in H\textsubscript{2}O/acetonitrile mixtures yields \textit{m/z} 586.2 (Figure 3B) and \textit{m/z} 570.2 ions (Figure 3E), respectively. These differences in \textit{m/z} values correspond to substitution of seven exchangeable H\textsuperscript{+} for seven D\textsuperscript{+} ions, plus one extra D\textsuperscript{+} ion to generate the positively charged molecular ion. This observation allows to exclude hydroxylation of the pyrenyl ring as a potential mechanism of O-atom addition to the dG\textsuperscript{Py} nucleoside, as suggested in our previous report (18).

**Circular dichroism spectra of the dG\textsuperscript{Py\*} nucleoside adduct**

Photooxidation of the 10\textit{S}(+)-\textit{trans-anti-[Py]-N}\textsuperscript{2}-dG to form the dG\textsuperscript{Py\*} product is associated with significant changes in the circular dichroism spectra as shown in Figure 4. However, the absorption spectra of the dG\textsuperscript{Py} and dG\textsuperscript{Py\*} are very close to one another, indicating that the Py residue is intact in the dG\textsuperscript{Py\*} oxidation product. To explain these dramatic changes in the CD spectra, we propose that the photooxidation of the dG\textsuperscript{Py} adduct induces a cleavage of the non-aromatic cyclohexenyl ring. The opening of the aliphatic ring leads to changes in the relative orientations of the guanosyl and the pyrenyl residues while maintaining nearly unchanged absorption spectra; nevertheless, the 3 nm blue shifts in the absorption maxima of dG\textsuperscript{Py\*} product suggest weaker Py – dG base stacking interactions (inset in Figure 4). The cyclohexenyl ring-opening leads to diminished orientation-dependent dipole-dipole interactions and thus a weakening of the CD spectra in the dG\textsuperscript{Py\*} adduct. In the non-oxidized dG\textsuperscript{Py} nucleoside adduct, extensive dipole-dipole interactions result in a strong exciton coupling between these two moieties and thus the strong CD spectrum of the (+)-\textit{trans-anti-[Py]-N}\textsuperscript{2}-dG adduct (23, 24). In this ring-opened structure, the guanosyl residue can assume different conformations because of the greater number of torsional degrees of freedom, in contrast to the intact (+)-\textit{trans-anti-[Py]-N}\textsuperscript{2}-dG adduct that is conformationally severely constrained by steric hindrance effects (5, 6).

**Rupture of the cyclohexenyl ring is required to form the dG\textsuperscript{Py\*} adduct**

Formation of the G\textsuperscript{Py\*} product from the parent G\textsuperscript{Py} adduct by photocleavage of the cyclohexenyl ring is a unique pathway that can account for all of the experimental observations: (1) The difference in mass of 16 Da between dG\textsuperscript{Py\*} and dG\textsuperscript{Py}, (2) The insertion of an O-atom from H\textsubscript{2}\textsuperscript{18}O without increasing the number of exchangeable protons, (3) The dramatic changes in CD spectra, and (4) The conservation of the intact G and the aromatic Py ring system in the dG\textsuperscript{Py\*} adduct. There are examples of photochemical reactions, which involve heterolytic C-C bond cleavage in primary photochemical reaction steps, e.g., the photodecarboxylation of arylacetate ions (25), photodeformylation of 9-fluorene and 9-xanthenemethanols (26), and the photo-retro-aldol type processes of nitrophenylethyl alcohols (27, 28). Photocexcitation of these compounds in aqueous solutions induces heterolytic cleavage of the benzylic bonds to form the carbanion and carbocation-equivalent fragments, which immediately react with water to form end products. For instance, the UV photolysis (300 – 350 nm) of 2-(4-nitrophenyl)-l-phenylethanol in aqueous solutions generates 4-nitrotoluene and benzaldehyde products arising from the heterolytic cleavage of the benzylic bond in the parent compound (27). Here, we propose that the photoinduced heterolytic cleavage of the “benzylic” C9-C10 and C7-C8 bonds can initiate the formation of the M+16 products (Figure 5). The molecules 1 and 2 can be generated by cleavage of the C9-C10 bond and O-atom addition to either C10 (1) or C9 (2), whereas the molecules 3 and 4 can be produced by cleavage of the C7-C8 bond followed by O-atom addition to C7 (3) or C8 (4), respectively. The molecules 1 – 4 have intact G and Py.
moieties, one additional O-atom (in the form of the carbonyl group), and the same number of exchangeable protons as the parent dGPy adduct. Additional support for the dGPy* cyclohexenyl ring cleavage was obtained by analysis of the fragmentation patterns of the M*+16 product observed in the positive product ion mode.

**Positive product ion spectra**

Representative positive product ion spectra of the dGPy* adduct are shown in Figure 6. Fragmentation of the molecular ion, [M+H]+ detected at m/z 586.2 induces the detachment of the sugar residue (−116 Da) to form the aglycone ion, [BH2]+ observed at m/z 470.1. The later ion is further fragmented by three pathways: (1) detachment of the pyrenyl residue to form the protonated guanine, [GH]+ at m/z 152.2 (29), (2) expulsion of H2O from the former cyclohexenyl ring to form the ion at m/z 451.1, and (3) cleavage of the pyrimidine ring of the guanine residue. The latter involves breakage of the C5 – C6 and N3 – C2 bonds to form the ion detected at m/z 394.2. This fragmentation mode of the pyrimidine ring has been detected in the 4-aminobiphenyl-N2-dG adducts (30). The above fragmentation pathways are shown in Figure 7 for structure 1 (Figure 5), which is the most probable photoproduct (see, below). Thus, the product ion spectra support our hypothesis that the G and Py residues remain intact in the dGPy* product and that the addition of an O-atom to the cyclohexenyl ring is associated with its cleavage.

In the case of the parent dGPy adduct, fragmentation of the pyrimidine ring in the aglycone ion involves cleavage of the N2 – C10 bond between the exocyclic NH2 group and the pyrenyl residue to form [GH]+ at m/z 152.2 and the pyrenyl fragment at m/z 303.1 (Figure S1, Supporting Information). In the case of 10S(+) -trans-anti-[Py]-N2-8-oxodG we found an alternative pathway of fragmentation of the pyrimidine ring in the aglycone ion, which involves breakage of the C6 – N1 and C4 – N3 bonds to form the ions detected at m/z 362.1 and m/z 359.1 (Figure S2). The further fragmentation of the protonated free base, [GH]+ (Figures 6, S1 and S2) involves cleavage of the C6 – N1 bond followed by a collapse of the ring-opened ion formed by two principal pathways: (1) expulsion of ammonia (NH3) to form the ion at m/z 135.1, and (2) expulsion of CH2N2 fragment (as cyanamide, NHCN or carbodiimide, HNCNH) to form the ion at m/z 110.1 (29). Assignment of the ions generated by fragmentation of the pyrenyl residues was made by comparison of the product ion spectra of the dGPy* adduct (Figure 6), the parent 10S(+) -trans-anti-[Py]-N2-dG adduct (Figure S1), the 10S(+) -trans-anti-[Py]-N2-8-oxodG adduct (Figure S2), and 7,8,9,10-tetrahydroxyltetrahydrobenzo[al]pyrene (Figure S3).

**Proposed mechanism dGPy* formation**

Based on these cumulative results, we propose that the two-photon excitation of the pyrenyl residues of free 10S(+) -trans-anti-[Py]-N2-dG nucleoside, or the same adduct embedded in oligonucleotides, induces a heterolytic cleavage of the “benzylic” C9-C10 (Figure 8) or C7-C8 bonds in the cyclohexenyl ring (25-27). In aqueous solutions the carbanion and carbocation-equivalent fragments formed in the primary photochemical step immediately react with water to form either the hydroxyalkyl-substituted amine 5 (Figure 8), or products with two terminal OH-groups (see, below). The hydroxyalkyl-substituted amine 5 is unstable and can either decay to form the intact guanine and aldehyde fragments, or undergo oxidation that results in adduct 1 containing the amide fragment instead of the amine in the parent dGPy. The similar example of the amine to amide transformation is the oxidation of 2-(benzylideneamino)-2′-deoxyadenosine to 2-benzamido-2′-deoxyadenosine (31). Our experiments have shown that O2 is not required for the generation of the dGPy* product (18) and the most likely oxidizing agents that can serve as the electron acceptors in this step are the dGPy radical cations that are generated by laser photoexcitation. In H218O solutions this pathway involves 18O-atom addition to C10 and the transformation of 5 to the dGPy*.
product 1 containing one $^{18}\text{O}$-atom (Figure 3A). However, the exact nature of the oxidant requires further investigation. This proposed pathway is supported by direct experiments: (1) the presence of one $^{18}\text{O}$-atom in the dG$^\text{Py}^*$ photoproduct that is derived from in H$_2^{18}\text{O}$ (Figure 3A), and (2) the photodetachment of the Py-residues and the recovery of the intact guanine bases (18, 21). In contrast, pathways that involve O-atom addition to C7, C8 or C9 to form products with two terminal OH-groups, are less favorable than those resulting in the formation of the hydroxyalkyl-substituted amine 5. The two terminal OH-groups are unstable and should rapidly transform to aldehyde groups, which can be further oxidized to carboxyl groups that result in products 2 – 4 (Figure 5). In H$_2^{18}\text{O}$ solutions these pathways should result in the insertion of two $^{18}\text{O}$-atoms (the first $^{18}\text{O}$-atom added after the heterolytic cleavage remains in the adduct with a probability of ~0.5 during formation of the aldehyde group, and the second $^{18}\text{O}$-atom is added during oxidation of the aldehyde to the carboxyl group). However, our experiments show that only one $^{18}\text{O}$-atom derived from H$_2^{18}\text{O}$ is present in the dG$^\text{Py}^*$ product (Figure 3A). Therefore, the formation of structures 2 – 4 containing carboxyl groups (Figure 5) is unlikely. Although the formation of adduct 1 can account for all of the experimental results, further experiments are required to achieve an unambiguous structural assignment. However, investigations along these lines were beyond the scope of this work.

The oxidation pathways depicted in Figure 8 limit the injection of holes into the DNA strand and thus diminish the yield of nucleobase oxidation at oligonucleotide sites distant from the site of the photosensitizer (18). Our results suggest that in DNA hole injection experiments and studies of hole migration in DNA, it is desirable to determine the fate of the photosensitizer moiety to ensure that the photofragmentation of the sensitizer itself does not yield any potentially mobile reactive intermediates that could also lead to secondary DNA damage.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations**

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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Py</td>
<td>pyrene</td>
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<td>dG$^\text{Py}$</td>
<td>10.5(+) trans-anti-[Py]-N$^2$-dG</td>
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<td>anti-BPDE</td>
<td>(+)-7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene</td>
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<td>2-amino-5-[(2-deoxy-β-D-erythropentofuranosyl) amino]-4H-imidazol-4-one</td>
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<td>8-oxodG</td>
<td>8-oxo-7,8-dihydro-2′-deoxyguanosine</td>
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References


Figure 1.
Photochemical damage of 10S (+)-trans-anti-[Py]-N²-dG adducts site-specifically positioned in the oligonucleotide sequence (18). dG₁Py⁺⁺ is M+16 lesion.
Yields of the M+16 product dG\textsuperscript{Py}\textsuperscript{*} resulting from the laser flash photolysis of the d(CAT\textsubscript{1}G\textsubscript{Py}\textsubscript{1}CG\textsubscript{2}TCCTAC) sequence and after its complete enzymatic digestion to the nucleoside level (from the data of Yun et al.\textsuperscript{(18)}), or the nucleoside adduct dG\textsubscript{Py}\textsubscript{,10S (+)-trans-anti-[Py]-N\textsuperscript{2}-dG} adduct (this work). The experiments were conducted in air-equilibrated phosphate buffer solutions (pH 7.0) and irradiation by a train of 355 nm laser flash pulses (4 mJ/cm\textsuperscript{2}, 10Hz) for 20 s.
Figure 3.
Positive ion spectra of the dGPy* adducts generated by photolysis of 10S (+)-trans-anti-[Py]-N²-dG. (A) The dGPy* adduct synthesized in H₂¹⁸O buffer solutions (mass M+18). (B) The dGPy* adduct synthesized in H₂¹⁶O buffer solutions (mass M+16). (C) The dGPy* adduct in D₂O. (D) The dGPy in D₂O. (E) The dGPy adduct in H₂¹⁶O (mass M). The spectra were recorded using 0.1% formic acid in H₂O/CH₃CN = 1 : 1 v/v mixtures (A, B and E), and 0.1% formic acid in D₂O/CH₃CN 1 : 1 v/v (C and D) mobile phases, respectively. In D₂O mobile phases, the m/z values of sodium adducts (*Na) are greater by 21 Da than those of the corresponding molecular ions.
Figure 4.
Figure 5.
Proposed structures of dGPy* adduct produced by the rupture of the cycloexynyl ring.
Figure 6.
Positive product ion spectra of the dGPy* adduct recorded at different fragmentor voltages (A) 90 V, (B) 130V, and (C) 190 V.
Figure 7.
Fragmentation pathways of molecular and daughter ions of the dGPy* adduct.
Figure 8.
Proposed mechanism of M+16 product formation.