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Analysis of Novel Intermediate of Guanine-Guanine Crosslink Produced in Reactions of One-Electron Oxidation of Guanine Derivatives by Using 8-Substituted 2´-Deoxyguanosines as Analog Compounds

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Abstract

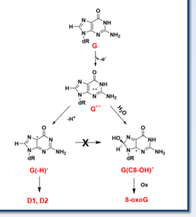
Oxidative damage to DNA has been implicated in a plethora of pathologies, such as cancer, neurodegenerative diseases, cardiovascular diseases, and aging. One-electron transfer (OET) plays a significant role in oxidative DNA damage in vivo. Guanine as the most oxidizable part of DNA is the major focus of studies on oxidation damage to DNA initiated by OET. Until recently, the pathway of guanine one-electron oxidation via its neutral guanine radical, G·, has been poorly studied. Our recent research has discovered a novel type of products of G· dimerization, D1 and D2, formed as a result of oxidation reaction of guanine derivatives, initiated by OET. A proposed reaction mechanism contains an early intermediate (Int1) generated by recombination of the two G· radicals. We were not able to isolate Int1, so that its role in the proposed reaction mechanism is only hypothetical. Literature data have reported that 8-arylamino-substituted guanosine (Guo) compounds can be oxidized to create structural analogs of D1 and D2. As a result, the original 8-substituted Guo compounds can serve as analogs of Int1. The goal of this work is therefore to confirm that Int1 is a precursor to D1 and D2 using the analogy approach.

8-arylamino- and 8-alkylamino-substituted Guo compounds were synthesized, purified by semipreparative HPLC, and their structures were confirmed by ^{1}H-NMR. Substituted oxidation products analogous to D1 and D2 were obtained from 8-substituted Guo analogs upon illumination the reaction mixture in the presence of $S_2O_8^{2-}$ as an oxidant and $Ru(II)(bpy)_3^{2+}$ as a photosensitizer at 470 nm. The products were purified by semipreparative HPLC, and their structures were confirmed by ^{1}H-NMR. The purified analogs of D1 were successfully tested for conversion into the D2 analog. Finally, the analogs of D2 were successfully tested for the reaction with primary amines to form 2-aminoimidozalone (Alz), in agreement with the mechanism characteristic of D2.

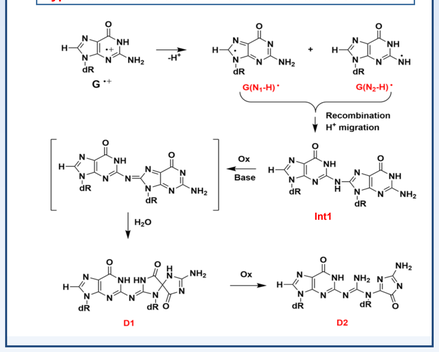
Background

One-Electron Oxidation of Guanine in DNA

- **One-electron oxidants**: complexes of transition metals (Fe(III), Cr(V)) $CO_3^{\bullet-}$, $ROO^{\bullet}$, $SO_4^{\bullet-}$, $Br_2^{\bullet-}$
- One-electron oxidation of guanine results in radical-cation $G^{\bullet+}$, which deprotonates to form a neutral guanine radical $G(-H)^{\bullet}$ in competition with its hydrolysis
- The hydrolysis product $G(C8\text{-}OH)^{\bullet}$ turns into 8-oxoguanine upon further oxidation while the fate of $G(-H)^{\bullet}$ remains largely unknown.
- $G(-H)^{\bullet}$ is known for its low reactivity and decays bimolecularly in the absence of reducing agents.



Hypothetical Reaction Scheme of Formation of D1 and D2

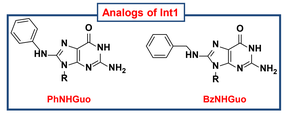


Aims

1. To synthesize 8-arylamino- and 8-alkylaminoguanosino derivatives of Int1 and to confirm their identity by NMR.
2.To optimize the conditions of reactions with several one-electron oxidants with the derivatives synthesized in Aim 1 to prepare analogs D1 and D2.
3.To isolate analogs of D1 and D2 and confirm their identity by NMR.
4. Isolated analogs of D1 will be tested for conversion into D2 analogs upon one-electron oxidation.
5. Isolated analogs of D2 will be tested for the reaction with primary amines to form 2-aminoimidozalone (Alz), in agreement with the mechanism characteristic of D2.

Experimental Methods

Analogs of Int1



Reaction of Formation of $SO_4^{\bullet-}$ by Photooxidation of $Ru(II)bpy_3^{2+}$ in the Presence of $S_2O_8^{2-}$

$$Ru(II)(bpy)_3^{2+} + h\nu \rightarrow Ru(II)(bpy)_3^{2+*}$$
$$Ru(II)(bpy)_3^{2+*} + S_2O_8^{2-} \rightarrow Ru(III)bpy_3^{3+} + SO_4^{\bullet-} + SO_4^{2-}$$

~ 2-5 mM substrates were photolyzed at room temperature at 470 nm in 50 mM phosphate buffer, pH 6.9, in the presence of 50 mM $Ru(II)(bpy)_3^{2+}$ (photosensitizer) and 10-20 mM $S_2O_8^{2-}$.

Other One-Electron Oxidants

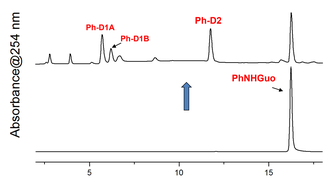
$Fe(III)(bpy)_3^{3+}$ reaction. 20 mM stock solution of tris(bipyridine)iron(III) (oxidizer) was prepared in dry MeCN (blue solution). Reaction was conducted at room temperature in 50 mM phosphate buffer, pH 6.9 in the presence of 4 mM oxidizer to form a red solution of tris(bipyridine)iron(II). To precipitate iron complexes, the reaction mixture was stirred with ion exchange resin until the solution became clear.

$Fe(CN)_6^{3+}$ reaction. 2.5 mM PhNHGuo, 5 mM $Fe(CN)_6^{3+}$, and 50 mM K_2CO_3 were mixed with stirring and then neutralized with acetic acid, followed by ion exchange resin until the solution became clear.

Reaction with Primary Amines

The reaction was conducted at 15 °C for 25 min in the presence of 0.2 M solution of ethanolamine (EA).

Representative Chromatograms of Formation of Analogs of D1 and D2 by Oxidation of PhNHGuo by $SO_4^{\bullet-}$



Photolysis of 5 mM solution of PhNHGuO in 50 mM phosphate buffer, pH 6.9 at 470 nm for 1.5 min in the presence of 50 mM $Ru(II)(bpy)_3^{2+}$ (photosensitizer) and 20 mM $S_2O_8^{2-}$(oxidizer).

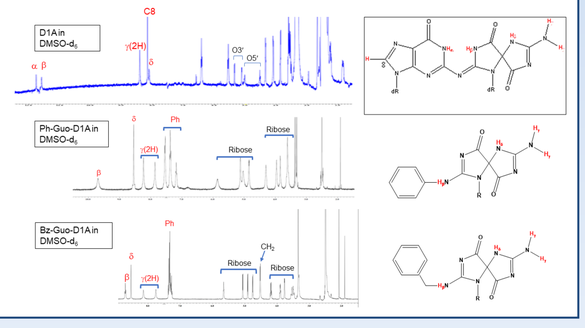
Chromatography Methods

HPLC Analysis. Gemini 4.6 mm×250 mm column (Phenomenex),1 mL/min flow rate, 30 °C, gradient elution (isocratic 40 mM ammonium acetate during the first 6 min, then 0-13% MeCN in the next 10 min).

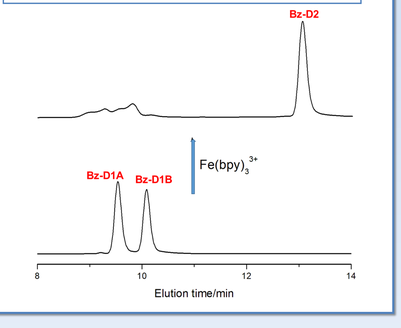
HPLC Purification. Gemini 10 mm×250 mm column (Phenomenex),3.5 mL/min flow rate, 30 °C, gradient elution (isocratic 40 mM ammonium acetate during the first 6 min, then 0-13% MeCN in the next 10 min). Isolation of D1 and D2 analogs from the reaction mixtures was performed by manually collecting the fractions containing these products, drying them in centrivap, and reconstitution in a small amount water or 0.1% acetic acid . The separation of D1 analog diastereomers and final purification of D2 analogs for the studies of their reactions were performed under the same conditions except for using 0.1% acetic acid instead of ammonium acetate as the mobile phase.

Results

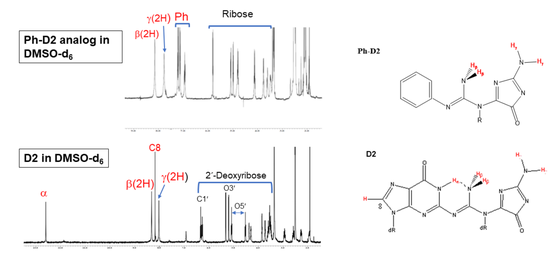
^{1}H-NMR and Structure of Aryl-D1 Analogs



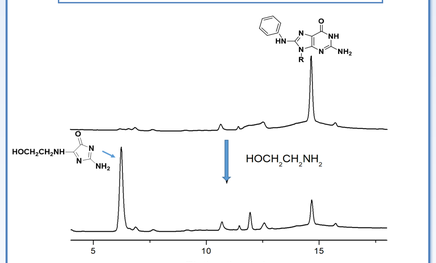
One-Electron Oxidation of Bz-Substituted Analogs of D1 Produces Bz-Substituted Analog of D2



^{1}H-NMR and Structure of Ph-D2 Analog



Reaction of Ph-Substituted Analog of D2 with Amines Produces 5-Aminoimidazolone (Alz)



Discussion

- One-electron oxidation of 8-alkyl and 8-arylaminoguanosines results in products that belong to the same structural type as the dimerization products (D1 and D2) formed by oxidation of guanosine and 2'-deoxyguanosine.
- These oxidation products display the same type of reactivity towards further oxidation converting D1 analog into D2 analog, and reaction with aliphatic primary amines as their guanosine-derived analogs.
- These results strongly suggest that the precursor to the guanine dimers is Int1, a guanine-guanine cross-link 8-(guanosine-N2-yl)guanosine, in which the bond is formed between the exocyclic nitrogen of one guanine moiety, and the C8-position of the other.

References

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Acknowledgements

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