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The Effects of Ultraviolet Radiation on Nucleoside Modifications in RNA

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Abstract

Ultraviolet radiation (UVR) is a known genotoxic agent. Although its effects on DNA have been well-documented, its impact on RNA and RNA modifications is less studied. By using *Escherichia coli* tRNA (tRNA) as a model system, we identify the UVA (370 nm) susceptible chemical groups and bonds in a large variety of modified nucleosides. We use liquid chromatography tandem mass spectrometry to identify specific nucleoside photoproducts under *in vitro* and *in vivo* conditions, which were then verified by employing stable-isotope labeled tRNAs. These studies suggest that the -amino or -oxy groups of modified nucleosides, in addition to sulfur, are labile in the oxidative environment generated by UVA exposure. Further, these studies document a range of RNA photoproducts and post-transcriptional modifications that arise because of UVR-induced cellular stress.

Graphical abstract

RNA contains >140 different naturally occurring nucleoside modifications,¹ and recent reports find that modified nucleosides in tRNA (tRNA) can vary in response to cellular stress conditions.^{2–7} We have an interest in understanding modification variations under particular

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Supporting Information

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Notes

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oxidative stress conditions such as ultraviolet radiation (UVR) exposure. The incident UVR on the earth's surface comprises 95% UVA (315–400 nm) and 5% UVB.⁸ The high incidence of UVA (20-fold higher than UVB), its oxidative potential through cellular photosensitizers,^{9–11} and its ability to reach melanocytes and dividing stem cells¹² could deliver multiple potential impacts to RNA.

While earlier work has shown that sulfur-containing modifications (*e.g.*, 2-thiouridine) are degraded and converted to 4-pyrimidinone upon H₂O₂-induced oxidative stress,¹³ affecting the structure and function of tRNAs,¹⁴ a comprehensive picture of UVA-induced changes in the nucleoside modifications of tRNAs is lacking. Such information is important as sublethal oxidative damage to coding and noncoding RNA is shown to contribute to neuronal cell death and neurodegenerative diseases such as epilepsy, atherosclerosis, and Alzheimer's and Parkinson's disease.¹⁵ We have conducted investigations to identify the affected RNA modifications and the photoproducts formed upon UVA exposure by liquid chromatography tandem mass spectrometry (LC-MS/MS). We report the identification of modifications beyond thiolations that are affected by UVA-induced stress under *in vitro* and *in vivo* conditions and postulate the sites of attack in these modifications.

To understand the oxidative environment generated by UVA exposure, we exposed *E. coli* total tRNA with or without riboflavin (RF, a photosensitizer) for 20 min and measured purine oxidation using LC-MS/MS following complete hydrolysis of tRNA. Without RF, UVA induced a small but reproducible increase of the two-electron oxidation product, 8-oxoguanosine (8-oxo-rG; Supporting Information Figure S1). The addition of 10 μ M RF generated the four-electron oxidation product, 5-guanidinohydantoin-ribonucleoside (Gh), whose level increased at 100 μ M RF but then was constant at higher concentrations of RF. Time-dependent UVA exposure of tRNA in the presence of 100 μ M RF revealed formation of 8-oxo-rG at 2 min and Gh after 2 min. These studies indicate that longer UVA exposure and higher RF concentrations yield primarily the four-electron product, Gh. A small percentage of adenosine oxidation was observed in the presence of RF following 2 min of exposure. The subsequent oxidation product, 5-formamidopyrimidine (Fapy-A), was detected after 10 min of exposure. Generation of Gh and Fapy-A were confirmed by employing ¹⁵N-labeled tRNA, where molecular ions at *m/z* 295.0943 corresponding to Gh (C₉O₆¹⁵N₅H₁₆, calculated *m/z* 295.0952, 3 ppm error) and *m/z* 291.0994 corresponding to Fapy-rA (C₁₀O₅¹⁵N₅H₁₆, calculated *m/z* 291.0999, 2 ppm error) were detected (Supporting Information Figure S2). These data suggest that RF and longer exposure to UVA generate conditions that could easily oxidize purines under *in vitro* conditions. These conditions did not yield any oxidation products of pyrimidines (data not shown).

After understanding the impact of UVA and RF on canonical nucleosides, we probed their effects on post-transcriptional modifications. The effects detected were characteristically different depending on the chemical composition of the modified nucleoside. UVA alone caused significant decreases in the abundances of mostly sulfur-containing modifications (2-thiocytidine-s²C, 4-thiouridine-s⁴U, uridine 5-oxyacetic acid-cmo⁵U, 5-methylaminomethyl-2-thiouridine-mnm⁵s²U, 5-carboxymethylaminomethyl-2-thiouridine-cmm⁵s²U, *N*⁶-isopentenyladenosine-i⁶A, and 2-methylthio-*N*⁶-isopentenyladenosine-ms²i⁶A; Figure 1, panel A; Supporting Information Figure S3, panel A). Similarly, RF alone

adversely affected the integrity of i⁶A, cmo⁵U, ms²i⁶A, and mnm⁵s²U, and UVA exposure accelerated their degradation (Figure 1, panel B; Supporting Information Figure S3, panel B). The UVA and RF combination led to significant decreases in 2-lysidine-k²C, N⁶-methyl-N⁶-threonylcarbamoyladenosine-m⁶t⁶A, N⁶-methyladenosine-m⁶A, N⁶-threonylcarbamoyladenosine-t⁶A, 5-carboxymethylaminomethyl-2'-O-methyluridine-cmm⁵Um, 1-methylguanosine-m¹G, and epoxyqueuosine/queuosine-oQ/Q under *in vitro* conditions (Figure 1). The impact on sulfur containing modifications is consistent with the previously reported reactive behavior with hydrogen peroxide.¹⁶ These modifications are known to maintain tRNA structural stability, as well as the accurate and efficient decoding of mRNA.^{17–22}

After identifying modified nucleosides affected by UVA and RF, we probed for potential modification photoproducts. We observed a progressive increase in 5-methylaminomethyluridine-mnm⁵U, 5-aminomethyluridine-nm⁵U, and 5-hydroxyuridine-ho⁵U in the UVA exposed sample. Although a small amount of mnm⁵U is detected in the unexposed sample, its abundance increased significantly upon UVA exposure (Supporting Information Figure S4). After 10 min, a decrease in mnm⁵U was accompanied by an increase of nm⁵U (Figure 2, panel A). The presence of these photoproducts is further supported by the data obtained with the ¹⁵N-labeled tRNA (Supporting Information Figure S5). Although these studies do not reveal the exact origin of these photoproducts, the dynamic changes in levels of mnm⁵U and nm⁵U over a period of increased UVA exposure suggest that nm⁵U could have originated from mnm⁵U, which itself is an oxidative product of mnm⁵s²U. Thus, nm⁵U might form through a series of chemical reactions involving dethiolation,¹⁴ oxidation, and demethylation (Figure 2, panel B).²³ Similarly, mo⁵U and ho⁵U were detected in the UVA exposed RNA samples. Although their source is unclear from this experiment, their detection in significant amounts in the UVR-exposed cmo⁵U standard (Supporting Information Figure S6) suggests that cmo⁵U could be their potential precursor.

To better understand the oxidative effects on post-transcriptional modifications observed under intracellular conditions, mid log phase *E. coli* K-12 cell culture was exposed to UVA for 1 and 2 h. Subsequent nucleoside analysis of isolated tRNA by LC-MS/MS revealed 8-oxo-rG as the predominant purine oxidation product. The four-electron oxidation product, Gh, was not detected under these conditions presumably due to a weaker oxidative environment inside UVA exposed cells compared to the *in vitro* conditions. The lack of any oxidized adenosine in the data also supports this conclusion.

Sulfur-containing modifications significantly decreased upon exposure of cells to UVA. Similarly, nonsulfur modifications such as cmo⁵U, i⁶A, Q, cmm⁵Um, k²C, ac⁴C (N⁴-acetylcytidine), t⁶A, and m⁶t⁶A significantly decreased following UVA exposure (Figure 3, panel A). All these modifications were adversely affected by *in vitro* exposure except ac⁴C, which surprisingly did not change in abundance for the *in vitro* studies. In general, longer exposure resulted in increased adverse effect on modification levels. Two methylated nucleosides, m¹G and m⁶A, which were degraded under *in vitro* conditions, were unaffected even after 2 h of exposure under *in vivo* conditions. These observations indicate that although there is some utility in extrapolating *in vitro* studies to *in vivo* conditions, no

generalizations about modified nucleoside behavior can be made. Interestingly, the level of the photo-oxidative product nm⁵U increased in abundance following cellular exposure to UVA (Figure 3, panel A), which is consistent with the *in vitro* observations (Figure 2, panel A).

Because sulfur-containing modifications were adversely affected by UVA consistently under *in vivo* and *in vitro* conditions, we explored the possibility of their association with oxidative stress effects. To test this, we exposed a mid log phase cell culture of *E. coli* strain *trmU* (deficient in the enzyme responsible for mnm⁵s²U biosynthesis)²⁴ to UVA. The tRNAs isolated from the UVA-exposed mutant strain exhibited higher levels of 8-oxo-rG compared to the K-12 wild type strain (Figure 3, panel B). These data suggest a protective role for this sulfur-containing modification against UVA-induced oxidative stress, although additional studies are required to confirm this possibility. The mutant strain also exhibited reduced viability as revealed by serial dilution studies compared to wild type following UVR exposure (Supporting Information Figure S7).

An evaluation of the affected modifications (Figure 4) indicate that -amino or -oxy groups, in addition to sulfur, are labile in the oxidative environment generated by UVA exposure. Detection of ho⁵U from cmo⁵U and progressively increasing levels of nm⁵U with a corresponding increase in UVA exposure are consistent with this interpretation of the data. However, an accurate determination of all the degradation products requires investigations with modified nucleoside standards and comparison against synthetic intermediates.

These studies open the opportunity to evaluate the impacts of UVA on tRNA structure, function, and global effects on cellular translation. Previous reports indicate that dethiolation of s²C at position 32 of tRNA(Arg^{ICG}) could lead to the removal of restrictions on translation of multiple codons, CGU, CGC, and CGA.²⁵ RNA duplexes containing an mnm⁵U-A base pair are less stable in the helical structure than those containing the mnm⁵s²U-A base pair.¹⁴ Similarly, the loss of other anticodon loop modifications, cmnm⁵s²U, cmo⁵U, cmnm⁵Um, or k²C (at position 34) and ms²i⁶A, i⁶A, t⁶A, and m⁶t⁶A (at position 37) of various tRNAs would severely impair the decoding function. Likewise, the loss of oQ can lead to read-through of the stop codon by tRNA(Tyr),²⁶ besides reducing the decoding efficiency of CAU (histidine) codons.²⁷

Studies using the mutant *E. coli* strain *trmU* reveal a greater abundance of oxidative damage products, implying a protective effect for sulfur containing modifications.^{20,21} Loss of a similar U34 modification mcm⁵s²U (5-methoxycarbonylmethyl-2-thiouridine)²⁸ and variants of ELP2²⁹ and ELP3³⁰ proteins (responsible for U34 thiolation) have been implicated in recessive neurodegenerative disease in humans. Although the data from *E. coli* and humans are coincidental, further studies will determine whether the unavailability of sulfur-containing modifications could lead to a loss of protective function and likely intensification of the recessive phenotypic effects by oxidative stress. This notion could be supported by the observation that the loss of thiolation at U34 of tRNAs leads to decreased viability upon nutrient starvation, oxidative stress, or exposure to higher temperatures in yeast and nematodes.^{31,32}

In summary, many post-transcriptional modifications are found to be vulnerable to degradation upon UVA-induced oxidative stress. Stable-isotope (^{15}N) labeled *E. coli* K-12 MG1655 tRNA was used to confirm the generation of some oxidative photoproducts. These studies identified-amino and -oxy groups, beyond thiolations, that are labile after UVA exposure under both *in vitro* and *in vivo* conditions. These studies also reveal that many structural modifications (such as m^7G , Gm, acp^3U , etc.) are insensitive to UVA exposure, suggesting tRNA tertiary interactions may be preserved while functional fine-tuning may suffer. This work provides new insights into the cellular lability of modified nucleosides, and future work will be able to expand these studies into eukaryotic and mammalian systems to determine whether cellular antioxidants in those organisms mitigate the effects found here with a simple bacterial system.

METHODS

Escherichia coli tRNA was procured from Roche Diagnostics and $^{15}\text{NH}_4\text{Cl}$ from Martek isotopes. Nuclease P1, Trireagent, and nucleoside test mix were obtained from Sigma-Aldrich. Riboflavin and all other chemicals were procured from Fisher Scientific.

E. coli Cell Culture

K-12 MG1655 and JW1119-1 (*E. coli* Genetic Stock Center) strains of *E. coli* were cultured in Luria–Bertani medium until mid log phase ($\text{OD}_{600} \sim 0.5$). The JW1119-1 strain harbors a deletion of the *trmU* gene, which is deficient in the modified nucleoside, $\text{mmn}^5\text{s}^2\text{U}$, found at position 34 of *E. coli* tRNA(Lys).²⁴

Isotope Labeled tRNA

^{15}N -labeled tRNAs were prepared by growing *E. coli* (K-12) in M9 minimal medium containing $^{15}\text{NH}_4\text{Cl}$ (>99.7% purity). Cells were harvested at mid log phase ($\text{OD}_{600} \sim 0.5$) and either exposed to UVA or used for purification of ^{15}N -labeled tRNA³³

UVA Exposure

E. coli tRNA ($5\ \mu\text{g}$; $0.5\ \mu\text{g}\ \mu\text{L}^{-1}$) or nucleoside standards (gift from Prof. McCloskey) were irradiated with UVA (370 nm peak wavelength, 360–400 nm range, $0.752\ \text{mJ}\ \text{cm}^{-2}$) in the presence of 0–1000 μM riboflavin (RF) for 0–30 min at RT (25 °C). The UVA source was made in-house by using Nichia STS-DAI-2749D LEDs (# 161031). Control or unexposed RNA samples were incubated in darkness in the presence of riboflavin for identical periods of time. For UVA exposure of tRNA *in vivo*, 2 mL of resuspended cells were exposed in a home-built unit designed to hold Petri plates. The LEDs (LED Engin catalog # LZ1–00UV00) used in this unit exhibited a 370 nm peak wavelength generating $3\ \text{mJ}\ \text{cm}^{-2}$.

Nucleoside Analysis

The UVA-exposed and unexposed tRNAs were hydrolyzed to ribonucleosides³⁴ and subjected to high resolution liquid chromatography coupled with mass spectrometry (HR-LC-MS) using a Vanquish UHPLC system coupled to an Orbitrap Fusion Lumos (Thermo Scientific) mass spectrometer. Nucleosides are identified based on their relative retention

time, accurate mass, and tandem mass spectral (MS/MS) behavior following collision-induced dissociation (CID).³⁴

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Changes in levels of post-transcriptional modifications of *E. coli* tRNA following UVA exposure. (A) Five micrograms of tRNA was exposed to UVA (0.752 mJ cm^{-2}) for 20 min at 0, 10, 100, and 1000 μM added riboflavin and analyzed by LC-MS for percent changes in levels of various modifications upon UVA exposure following normalization with the corresponding canonical nucleosides. (B) Effect of UVA exposure period on modifications. Five micrograms of tRNA was exposed to UVA (0.752 mJ cm^{-2}) for 0–30 min in the presence of 100 μM riboflavin, and analyzed for changes in modification levels by LC-MS. For the sake of clarity, the representative data of the mean of five replicates is shown without the error bars.

Figure 2.

Time-dependent UVA photoproduct generation. (A) Comparative analysis of the levels of mnm⁵U and nm⁵U with increasing UVR exposure. The percentage changes in levels of mnm⁵U and nm⁵U as a fraction of uridine were computed and plotted against each UVA exposure period. (B) Proposed mechanism for the conversion of mnm⁵s²U into mnm⁵U and nm⁵U involving dethiolation, oxidation, and demethylation is shown.

Figure 3.

LC-MS analysis of UVA-induced effects on tRNA modifications under *in vivo* conditions.

(A) Changes in tRNA modification levels following UVA exposure of *E. coli* cells. Each modification is expressed as a ratio of the corresponding signal in the exposed versus unexposed sample. The *P* values for each modified nucleoside (saving m¹G and m⁶A) as determined by Student's *t* test varied by 0.008547 (cmnm⁵Um, indicated by asterisk) or less. (B) Altered levels of guanosine oxidation following UVA exposure of mnm⁵s²U-deficient *E. coli* strain. Fold change in 8-oxo-rG level in tRNA isolated from UVA (3 mJ cm⁻², 2 h) exposed cells over unexposed cells is plotted for wild-type (striped bar) and mutant *trmU* (white bar). The signal for 8-oxo-rG was normalized to its canonical G in each sample. Data represent mean $\pm$ SD values of five biological replicates. Asterisk denotes the *P* value (0.004137) as determined by Student's *t* test.

Figure 4. Post-transcriptional RNA modifications affected by UVA stress. Potential sites of oxidative attack on each nucleoside modification¹ are shown by an arrow.