Published in final edited form as:
Chem Res Toxicol. 2011 November 21; 24(11): 1830–1832. doi:10.1021/tx200410x.

Photoactivated Uranyl Ion Produces Single Strand Breaks in Plasmid DNA

Shannon A. George, **Aaron M. Whittaker**, and **Diane M. Stearns***
Department of Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ 86011, United States

Abstract

Uranium is an important emerging toxicant whose use has outpaced the rate at which we are learning about its health effects. One unexplored pathway for uranium toxicity involves the photoactivation of uranyl ion by UV light to produce U^{5+} and oxygen radicals. The purpose of this study was to provide "proof of principle" data by testing the hypothesis that coexposures of DNA to uranyl acetate and UVB irradiation should produce more DNA strand breaks than individual exposures. Results supported the hypothesis and suggest that investigations of uranium toxicity be expanded to include skin as a potential target organ for carcinogenesis, especially in populations with high uranium and high UV radiation exposures.

Uranium is an important emerging toxicant whose use has outpaced the rate at which we are learning about its health effects, which complicates efforts to provide meaningful risk assessment. Uranium may be classified into three categories, natural, enriched, and depleted, based on differences in relative amounts of its three major radioactive isotopes: 238-U (abundance 99.3%, $t_{½} = 4.47 \times 10^9$ yr), 235-U (0.72%, $t_{½} = 7.04 \times 10^8$ yr), and 234-U (0.006%, $t_{½} = 2.46 \times 10^5$ yr).[1] Enriched uranium contains higher relative amounts of the 235-U isotope, which undergoes nuclear fission, and is thus used in nuclear weapons. Depleted uranium (DU) is the form that remains after the 235-U isotope is extracted for nuclear fission reactions and weapons. DU is thus less radioactive because it is relatively higher in the 238-U isotope. DU is predominantly used in the military for armor-piercing munitions and for lining tanks.

The chemical mechanisms of uranium toxicity are less well understood than the radiological mechanism, which makes it difficult to fully assess the impacts of uranium exposures. Contentious issues that would be aided by more research include political and legal discussions surrounding past and future mining practices for natural uranium,[2,3] debates regarding the effects of DU exposures on military personnel and civilians,[4,5] and proposed governmental bans of DU-containing munitions.[6] In agreement with this lack of data, the National Research Council has recently issued a report on the health risks of DU in military personnel, with the conclusion that "the committee recommends research into whether there is a chemical mechanism of uranium carcinogenesis." [7]

The most thoroughly studied target organs for uranium toxicity and carcinogenesis are lung[8–10] and kidney;[11–13] however, dermal absorption is also a major route of exposure.[14–17] Absorption of uranium through the skin has the potential to provide toxic

*Corresponding Author: Tel: (928)523-4460. Fax: (928) 523-8111. diane.stearns@nau.edu.

Supporting Information Available. Experimental procedures and supplementary figures. This information is available free of charge via the Internet at http://pubs.acs.org.

levels of uranium in the body after periods of only minutes to hours,[17] and uranium can be retained in the epidermis.[16]

One potential mode of action for uranium toxicity that has not been explored is the ability of uranyl ion to be activated by UV light. The first observation of the sensitivity of uranium to light was reported in 1805, when Buchholz described the decomposition of uranium sulfate in the presence of sunlight.[18] It has since found use as a chemical catalyst for the oxidation of a range of organics.[19] Nielsen later adapted the photoactivation of uranyl ion by UVA light for use as a molecular technique for DNA foot printing.[20] Thus uranyl photochemistry has been developed as a laboratory tool; however it has not been investigated as a mode of action for uranium toxicity.

The photoactivation of uranyl ion is driven by the absorption of UV light at ~300–420 nm[20] into the U=O bond which causes excitation of the electron pair, essentially forming U^{5+} and a uranium-bound oxygen radical[19] ($*UO_2^{2+}$, Scheme 1), which undergoes reduction by, for example, H atom abstraction from an organic substrate (Eqn. 1). Regeneration of the uranyl ion can occur through reduction of molecular oxygen (Eqn. 2). This photoactivation is a chemical process that is independent of the radioactivity of uranium, and provides another potential pathway for cellular toxicity, especially for situations resulting in dermal exposures.

Previous work in our laboratory has shown that cells exposed to DU as uranyl acetate (UA) generate DNA strand breaks,[21] uranium-DNA adducts,[21] and mutations[22] through chemical (non-radiological) mechanisms. It is well established that UVB light (290–320 nm) produces cyclobutane primidine dimers and pyrimidine(6–4)pyrimidone photo-products as major DNA lesions at dipyrimidine sites.[23] The purpose of this study was to test the hypothesis that if uranyl ion is photoactivated to produce U^{5+} and bound oxygen radicals, then pre-incubation of uranyl ion with DNA followed by UVB irradiation should produce more DNA strand breaks than individual exposures to uranyl ion and UVB light.

Our hypothesis was tested by exposing pBR322 plasmid DNA to 1.00 mM UA for 30 min, followed by exposure to UVB light at intensities of 18, 35, or 53 mJ/cm^2. Plasmid DNA was subjected to gel electrophoresis, and the increases in single strand breaks were quantified from digital images. Exposure of DNA to either UA or UVB light alone did not produce statistically significant plasmid relaxation. However, incubation of DNA with UA followed by exposures to UVB light did produce significant levels of DNA strand breaks (Figure 1).

The combined effect of UA and UVB light on DNA strand breaks was synergistic. DNA exposed to UA alone had ~8% more DNA relaxation than background relaxation in control DNA, or 1.6-fold more above background, a difference that was not statistically significant. UVB exposure alone produced ~3–4% more plasmid relaxation than control DNA, or 1.3-fold more above background, which was also not statistically significant. However, combined exposures of UA and UVB light resulted in increases of 67–80% for plasmid relaxation, or increases in relaxation of 6 to 7-fold above background, which was statistically significant. Additive formation of DNA strand breaks would have resulted in 38+1 % relaxation whereas the observed plasmid relaxation was 2.1–2.4-fold higher than additive.

The effect of varying the uranyl ion concentration was measured for plasmid DNA exposed to 35 mJ/cm^2 UVB light. Data showed that incubating plasmid DNA with 0.040, 0.20, or 1.00 mM UA concentration followed by exposure to 35 mJ/cm^2 UVB light resulted in 1.5, 3.1, and 4.3-fold higher levels of plasmid DNA relaxation relative to UVB light alone (Figure 2).

The effect of buffer was explored by comparing strand breaks generated by incubation of DNA with UA and UVB light in plasmid DNA buffered by 25.0 mM ACES, MOPS, or MOPSO at pH 7.0, 37 °C. There were no significant differences among buffers (Supporting Information).

As a control reaction, the activity of UA was compared to that of arsenite ion (As(III)). As(III) has been shown to act as a cocarcinogen with UV radiation for the induction of non-melanoma skin cancers,[24] presumably through mechanisms including inhibition of DNA repair; however, not by photoactivation of arsenite ion. As expected, As(III) did not produce DNA strand breaks in the presence or absence of UVB light (Supporting Information).

The concentrations of UA used in this study were higher than the U drinking water limits set by the US Environmental Protection Agency (30 μg/L, 0.13 μM) and the World Health Organization (15 μg/L, 0.063 μM). However, the lowest active dose in these studies, 200 μM UA, or 48 mg/L (Figure 2), is equivalent to the highest level of uranium measured in contaminated sites at Oak Ridge National Laboratory, 50 mg/L.[25] It is only 5-fold higher than uranium levels of 10.1 mg/L reported in ground water in South Carolina.[13] It is 14 to 17-fold higher than levels of 3,410 μg/L and 2,848 μg/L, reported for ground water in Finland[26] and Shiprock, NM,[27] respectively. Furthermore, the relatively high levels in this study were chosen based on limits of detection for this in vitro experimental system. A mammalian organism would not require the same percentage of DNA damage for mutagenicity or tumorigenicity.

In summary, uranium is known to be absorbed through the skin. Animal experiments have established that dermal absorption can produce toxic internal levels of uranium. Uranium can bind to DNA. Uranyl ion is known to be photoactivated by UV radiation to produce U^{5+} and bound oxygen radical. The results of the current study showed that combined exposures to uranyl ion and UVB radiation were more DNA damaging than either exposure alone. From known mechanisms, it may also be proposed that the spectrum of DNA damage will be different than that produced by either agent alone, a hypothesis that we are currently investigating. This study provides “proof of principle” data to support reexamination of the current paradigm that uranium is predominantly a radiological mutagen. These observations support expansion of the focus of uranium toxicity to include skin as a potential target organ for carcinogenesis, especially in populations with high uranium and UV radiation exposures, for example in Australia and the Southwestern US, where uranium is mined and processed, and in the Middle East, where veterans and civilians have been exposed to DU from military operations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources

This research was supported by NIH Grants U54CA096320 and ES019703 (DMS). Ms. George was supported through the STEP Program within the Partnership for Native American Cancer Prevention (NACP), NIH Grant U54CA143925. Mr. Whittaker was supported through a Hooper Undergraduate Research Fellowship at Northern Arizona University.

ABBREVIATIONS

ACES N-(2-acetamido)-2-aminoethanesulfonic acid

DU	depleted uranium
MOPS	3-(N-morpholino)propanesulfonic acid
MOPSO	3-(N-morpholino)-2-hydroxypropanesulfonic acid
UA	uranyl acetate
UN	uranyl nitrate

References

1. Bleise A, Danesi PR, Burkart W. J Env Rad. 2003; 64:93–112.
2. Panikkar B, Brugge D. Account Res. 2007; 14:121–153. [PubMed: 17844786]
3. Saint-Pierre S, Kidd S. Health Phys. 2011; 100:39–45. [PubMed: 21399410]
4. Bertell R. Int J Health Serv. 2006; 36:503–520. [PubMed: 16981628]
5. Brown M. Philos Trans R Soc Lond B 36. 2006; 1:649–679. [PubMed: 16687269]
6. Borrmann R. Med Confl Surviv. 2010; 26:268–280. [PubMed: 21314080]
7. NRC. Review of Toxicological and Radiological Risks to Military Personnel from Exposure to Depleted Uranium During and After Combat. The National Academies Press; Washington, D.C: 2008. p. 95
8. Hornung RW. Occup Med. 2001; 16:331–344. [PubMed: 11319055]
9. Canu IG, Ellis ED, Tirmarche M. Health Phys. 2008; 94:1–17. [PubMed: 18091147]
10. Leuraud K, Schnelzer M, Tomasek L, Hunter N, Timarche M, Grosche B, Kreuzer M, Laurier D. Radiat Res. 2011; 176:375–387. [PubMed: 21714633]
11. Arzuaga X, Rieth SH, Bathija A, Cooper GS. J Toxicol Environ Health Part B. 2010; 13:527–545.
12. Vacquier B, Caer S, Rogel A, Feuprier M, Tirmarche M, Luccioni C, Quesne B, Acker A, Laurier D. Occup Environ Med. 2008; 65:597–604. [PubMed: 18096654]
13. Wagner SE, Burch JB, Bottai M, Puett R, Porter D, Bolick-Aldrich S, Temples T, Wilkerson RC, Vena JE, Hebert JR. Cancer Causes Control. 2011; 22:41–50. [PubMed: 21080052]
14. de Rey BM, Lanfranchi HE, Cabrini RL. Environ Res. 1983; 30:480–491. [PubMed: 6832127]
15. Lopez R, Diaz Sylvester PL, Ubios AM, Cabrini RL. Health Phys. 2000; 78:434–437. [PubMed: 10749526]
16. Petitot F, Moreels AM, Paquet F. Drug Chem Toxicol. 2010; 33:316–324. [PubMed: 20429805]
17. Spagnul A, Bouvier-Capely C, Phan G, Landon G, Tessier C, Suhard D, Rebière F, Agarande M, Fattal E. Eur J Pharm Biopharm. 2011; 79:258–267. [PubMed: 21620969]
18. Described in Fay H. J Am Chem Soc. 1896; 18:269–289.
19. Mao, Y.; Bakac, A. J Phys Chem A. Vol. 101. 1997. p. 7929-7933.and references therein
20. Nielsen PE. Methods Mol Bio (NY). 2009; 543:87–96.
21. Stearns DM, Yazzie M, Bradley AS, Coryell VH, Shelley JT, Ashby A, Asplund CS, Lantz RC. Mutagenesis. 2005; 20:417–423. [PubMed: 16195314]
22. Coryell VH, Stearns DM. Mol Carcinog. 2006; 45:60–72. [PubMed: 16299811]
23. Ikehata H, Ono T. J Radiat Res. 2011; 52:115–125. [PubMed: 21436607]
24. Rossman TG, Uddin AN, Burns FJ. Toxicol Appl Pharmacol. 2004; 198:394–404. [PubMed: 15276419]
25. Wu WM, Carley J, Fienen M, Mehlhorn T, Lowe K, Nyman J, Luo J, Gentile M, Rajan R, Wagner D, Hickey RF, Gu B, Watson D, Cirpka O, Kitanidis PK, Jardine PM, Criddle CS. Environ Sci Technol. 2006; 40:3978–3985. [PubMed: 16830571]
26. Prat O, Vercouter T, Ansoborlo E, Fichet P, Perret P, Kurttio P, Salonen L. Environ Sci Technol. 2009; 43:3941–3946. [PubMed: 19544911]
27. Chang YJ, Peacock AD, Long PE, Stephen JR, McKinley JP, Macnaughton SJ, Hussain AK, Saxton AM, White DC. Appl Environ Microbiol. 2001; 67:3149–3160. [PubMed: 11425735]

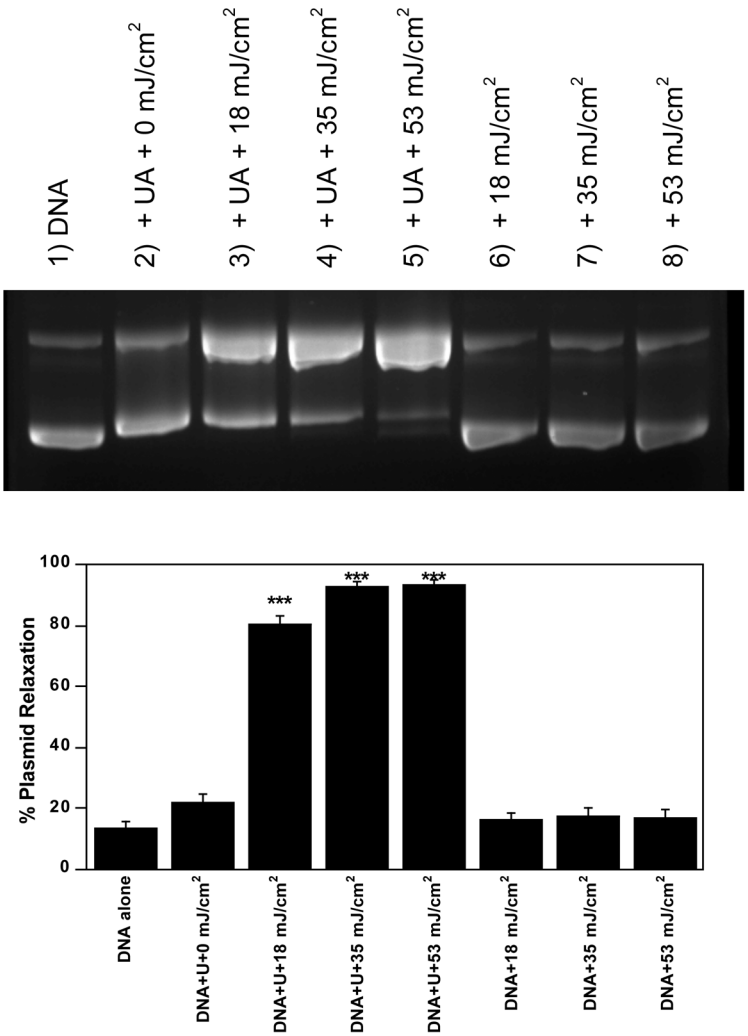

Figure 1.

(Upper) Representative gel showing generation of plasmid DNA strand breaks with 1.00 mM UA and increasing irradiation times in 25.0 mM ACES (pH 7.0, 37 °C). pBR322 DNA was incubated with UA for 30 min in the dark prior to irradiation with UVB at 18, 35, or 53 mJ/cm^2. **(Lower)** Quantification of DNA strand breaks for reaction conditions described in (A). Data represent mean+SEM for n=8 experiments. ***Plasmid relaxation for each reaction of UA+UVB was significantly greater than DNA alone, UA in the absence of UVB, and UVB in the absence of UA (ANOVA, P<0.0001).

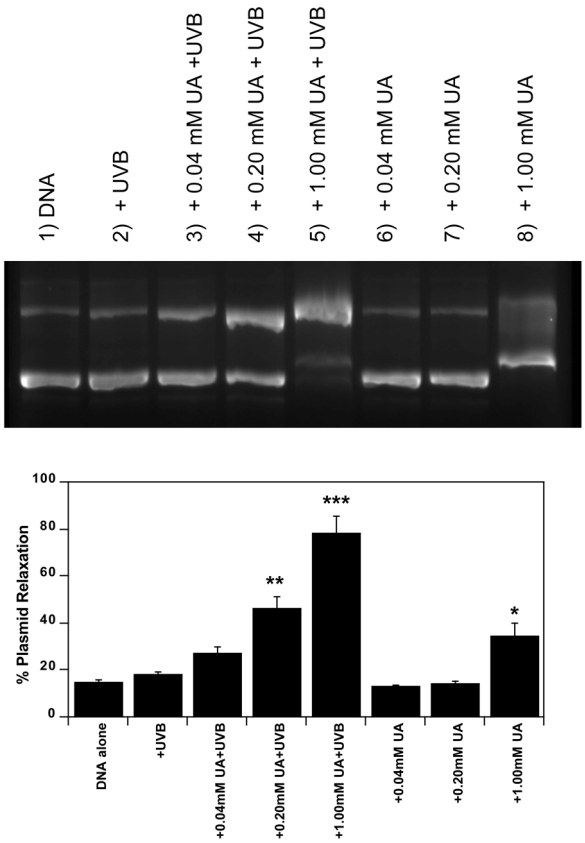

Figure 2.

(Upper) Representative gel showing generation of plasmid DNA strand breaks with increasing concentrations of UA+35 mJ/cm^2 UVB irradiation in 25.0 mM ACES (pH 7.0, 37 °C). pBR322 DNA was incubated with UA for 30 min in the dark prior to irradiation with UVB. **(Lower)** Quantification of DNA strand breaks for reaction conditions described in (A). Data represent mean+SEM for n=4 experiments. **Plasmid relaxation for reactions of 0.20 mM UA+UVB irradiation was significantly greater than that of both DNA alone and 0.20 mM UA–UVB (ANOVA, $P<0.001$). ***Plasmid relaxation for reactions of 1.0 mM UA+UVB irradiation was significantly greater than that of both DNA alone and 1.0 mM UA–UVB (ANOVA, $P<0.0001$). *Plasmid relaxation for 1.0 mM UA was slightly greater than for DNA alone (ANOVA, $P<0.05$).

$$^{*}UO_2^{2+} + RH \longrightarrow UO_2^{+} + R^{\bullet} + H^{+} \quad (1)$$

$$2UO_2^{+} + O_2 + 2H^{+} \longrightarrow 2UO_2^{2+} + H_2O_2 \quad (2)$$

Scheme 1.
Reactivity of Photoactivated Uranyl Ion