Published in final edited form as:

J Am Chem Soc. 2011 February 23; 133(7): 2148–2150. doi:10.1021/ja111585c.

A New Trick (Hydroxyl Radical Generation) For An Old Vitamin (B₁₂)

Thomas A. Shell and David S. Lawrence

Department of Chemistry, Division of Medicinal Chemistry and Natural Products, Department of Pharmacology University of North Carolina, Chapel Hill, NC 27599

Abstract

Photolysis of hydroxocobalamin in the presence of plasmid DNA (pBR322) results in DNA cleavage. Temporal control of hydroxyl radical production and DNA strand scission by hydroxocobalamin was demonstrated using a 2-deoxyribose assay and a plasmid relaxation assay, respectively. The light-driven hydroxocobalamin-mediated catalytic formation of hydroxyl radicals was demonstrated using radical scavenging studies of DNA cleavage and via recycling of a hydroxocobalamin-resin several times without loss of efficacy.

Hydroxyl radicals ($\cdot\text{OH}$) have been used to assess DNA structure¹ and to elucidate DNA-binding sites for proteins² and small molecules.³ An example of the Fenton reaction, in which $\text{Fe(II)}\cdot\text{EDTA}$ reduces H_2O_2 , is the most commonly used method to generate $\cdot\text{OH}$. However, for intracellular purposes, a limitation of this method is the inability to precisely control when the reaction starts and stops. A Fenton-like reaction utilizing a caged Cu(II) ion that can be released by photolysis of the ligand cage has been reported.⁴ This strategy furnishes control over the initiation of the Fenton reaction, albeit not the termination step. Several light-mediated “Fenton reagents” have been developed.⁵ In contrast to the Fenton reaction, the majority of these compounds have the disadvantage of stoichiometric radical production. TiO_2 does catalytically produce $\cdot\text{OH}$ upon exposure to light,⁶ however since TiO_2 induces intracellular oxidative damage in the absence of light,⁷ it cannot be used in a light-dependent fashion in cell-based studies. By contrast, synchrotron X-ray⁸ and γ ⁹ irradiation can be used to precisely initiate and terminate $\cdot\text{OH}$ production. Although these methods are advantageous for their ability to provide time resolved information, they are not widely available and they cannot be used to direct oxidative damage to specific intracellular or molecular sites.

Cobalamins, a class of compounds of which Vitamin B₁₂ is a member, could be useful for generating radical species under cellular conditions. For example, photolysis of the cobalt-carbon bond in methylcobalamin (MeCbl ; **1** where $\text{X} = \text{Me}$) generates cob(II)alamin ($\text{B}_{12\text{R}}$) and methyl radical ($\cdot\text{CH}_3$) (Scheme 1A).¹⁰ Based on this precedent, we wondered whether hydroxocobalamin ($\text{B}_{12\text{a}}$ where $\text{X} = \text{OH}$) could undergo an analogous reaction to generate $\cdot\text{OH}$ and $\text{B}_{12\text{R}}$ (Scheme 1B). In the presence of oxygen, the latter species is rapidly oxidized to regenerate $\text{B}_{12\text{a}}$.¹⁰ We therefore investigated the possibility that the combination of light and $\text{B}_{12\text{a}}$ could be used to oxidatively damage biomolecules via the generation of $\cdot\text{OH}$.

tshell@email.unc.edu and lawrencd@email.unc.edu.

Supporting Information Available: Figures S1-S22 and procedures are available free of charge via the internet at <http://pubs.acs.org>.

Initial studies employed a plasmid relaxation assay to observe the $\cdot\text{OH}$ -mediated conversion of circular, supercoiled DNA (Form I) to relaxed, circular DNA (Form II). A Pyrex filter ($>300\text{ nm}$) was used to photolyze $\text{B}_{12\text{a}}$ in the presence of pBR322 DNA. Analysis by agarose gel electrophoresis revealed a band corresponding to Form II DNA, but only from the light-exposed reaction mixtures containing $\text{B}_{12\text{a}}$ (Fig. S1). By contrast, light in the absence of $\text{B}_{12\text{a}}$ or $\text{B}_{12\text{a}}$ in the absence of light, does not result in significant DNA damage.

DNA cleavage by $\text{B}_{12\text{a}}$ does not occur in the absence of O_2 , an observation consistent with a radical-mediated mechanism of DNA damage (Fig. S3).¹¹ In addition, the $\cdot\text{OH}$ scavengers sorbitol¹² and sodium benzoate,¹³ efficiently prevent DNA cleavage (Fig. S5), thereby supporting the notion that damage is mediated by $\cdot\text{OH}$. The light-dependence of DNA cleavage was assessed by photolyzing $\text{B}_{12\text{a}}$ for various time periods followed by incubation in the dark (Fig. S8). Short photolysis periods followed by dark incubation resulted in significantly less strand scission than photolyzing for the same total time period, demonstrating that the species formed by the photolysis of $\text{B}_{12\text{a}}$ does not continue to damage DNA without continued exposure to light.

We employed an established $\cdot\text{OH}$ assay to assess light-mediated production of $\cdot\text{OH}$.¹⁴ Deoxyribose is attacked by $\cdot\text{OH}$, generating malondialdehyde that, upon heating with thiobarbituric acid (TBA), produces a chromophore (532 nm).¹⁵ Unfortunately, we found that the presence of $\text{B}_{12\text{a}}$ during the heating step interferes with the assay. Therefore, $\text{B}_{12\text{a}}$ was immobilized on an aminebearing resin (**2**) using 1,1'-carbonyldi-(1,2,4-triazole) (Fig. S10). Deoxyribose was incubated with the $\text{B}_{12\text{a}}$ -resin under photolytic and non-photolytic conditions and the resin was subsequently removed prior to the heating step. The light-driven nature of $\text{B}_{12\text{a}}$ -resin-mediated $\cdot\text{OH}$ production is evident by the time-dependent production of the TBA-malondialdehyde chromophore in the presence (Fig. 1, average absorbance increase per period: 0.104), but not in the absence (average absorbance increase per period: 0.003), of light.

Photolysis of the Co-methyl bond in MeCbl generates Co(II)-cobalamin and $\cdot\text{CH}_3$ (Scheme 1A). The former, upon oxidation, produces $\text{B}_{12\text{a}}$,¹⁰ which should generate DNA damaging $\cdot\text{OH}$ under photolytic conditions (Scheme 1B). However, $\cdot\text{CH}_3$ is also capable of causing DNA strand scission.¹⁶ We assessed the relative contribution of these two radical species to DNA damage using sorbitol and sodium benzoate ($\cdot\text{OH}$ traps) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO; a carbon-centered radical trap¹⁷). TEMPO does not have an appreciable effect on the ability of MeCbl to damage DNA, but sorbitol and sodium benzoate almost completely protect DNA from damage (Fig. S11). Therefore, the initial stoichiometric production of $\cdot\text{CH}_3$ is small compared to that of the $\text{B}_{12\text{a}}$ -mediated generation of $\cdot\text{OH}$.

The notion that $\text{B}_{12\text{a}}$ catalytically produces $\cdot\text{OH}$ is supported by radical trapping studies of DNA damage by re-using the $\text{B}_{12\text{a}}$ -resin **2** several times without loss in its capacity to damage deoxyribose (Fig. S13). Furthermore, a $\text{B}_{12\text{a}}$ concentration less than the pBR322 concentration was used to demonstrate catalytic production of strand scission events. Based on the number of observable strand scission events, $\text{B}_{12\text{a}}$ induced a minimum of 20 damaging events per molecule during 90 min of light exposure (Fig. 2); an underestimate since plasmid relaxation assays do not visualize all DNA nicks. The amount of DNA damage per molecule increased with longer exposure to light. A band corresponding to linear DNA (form III) resulting from nonrandom double-strand cleavage was observed (Figs. S15-16),¹⁸ indicative of two radicals originating near each other in time and in space.

A $\text{B}_{12\text{a}}$ -spermine conjugate **3** was synthesized (Fig. S17) and proved useful for directing damage to DNA in an environment with a high concentration of a hydroxyl radical trap.

Conjugate **3** forms a DNA complex with an apparent binding constant ($K_{\text{app}} = 1.5 \times 10^8 \pm 3.4 \times 10^6 \text{ M}^{-1}$) similar to the reported value for spermine ($5.9 \times 10^8 \text{ M}^{-1}$).¹⁹ The B_{12a}-spermine conjugate induces DNA damage more effectively than B_{12a} (Fig. S20). More importantly, 10000 equiv. of sorbitol failed to significantly inhibit DNA damage induced by **3**, whereas only 100 equiv. of sorbitol significantly inhibited strand scission due to B_{12a} (Fig. S22), suggesting that **3** generates $\cdot\text{OH}$ near the DNA **3** it is bound to.

Light-dependent $\cdot\text{OH}$ production by B_{12a} enjoys several characteristics that make it potentially useful as a generator of $\cdot\text{OH}$ for biological studies. Light is increasingly employed in modern cell biology to control intracellular processes via cell-embedded photosensitive bioactive reagents.²⁰ The combination of light and light-sensitive reagents provides the investigator with exquisite temporal control over cellular biochemistry. Spatial control of oxidative damage is afforded by the addition of a binding moiety to B_{12a} because the highly reactive $\cdot\text{OH}$ is generated proximal to the target biomolecule. In addition, light-induced radical generation with B_{12a} is catalytic, whereas radical generation by other systems is stoichiometric. Future research will focus on conjugating recognition moieties to B_{12a} to direct oxidative damage to specific macromolecules and organelles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the NIH (CA079954).

References

1. Tullius, TD. Radiation Research. Moriarty, M., editor. Vol. 2. Allen Press; Lawrence, KS: 2000. p. 333
2. a Jain SS, Tullius TD. Nat. Protoc. 2008; 3:1092. [PubMed: 18546600] b Frazee RW, Taylor JA, Tullius TD. J. Mol. Biol. 2002; 323:665. [PubMed: 12419257] c Zaychikov E, Schickor P, Denissova L, Heumann H. Methods Mol. Biol. 2001; 148:49. [PubMed: 11357607]
3. Trauger JW, Dervan PB. Methods Enzymol. 2001; 340:450. [PubMed: 11494863]
4. Ciesinski KL, Haas KL, Dickens MG, Tesema YT, Franz KJ. J. Am. Chem. Soc. 2008; 130:12246. [PubMed: 18714999]
5. a Haruna S, Kuroi R, Kajiwaru K, Hashimoto R, Matsugo S, Tokumaru S, Kojo S. Bioorg. Med. Chem. Lett. 2002; 12:675. [PubMed: 11844698] b Adam W, Cadet J, Dall'Acqua F, Epe B, Ramaiah D, Saha Moeller CR. Angew. Chem., Intl. Ed. Eng. 1995; 34:107. c Matsugo S, Kawanishi S, Yamamoto K, Sugiyama H, Matsuura T, Saito I. Angew. Chem., Int. Ed. Engl. 1991; 30:1351. d Aveline BM, Matsugo S, Redmond RW. J. Am. Chem. Soc. 1997; 119:11785. e Tobin D, Arvanitidis M, Bisby RH. Biochem. Biophys. Res. Commun. 2002; 299:155. [PubMed: 12435402] f Kitano H, Maeda Y, Furukawa K, Yamamoto T, Izumida R, Matsugo S. Photochem. Photobiol. 1995; 62:809. [PubMed: 8570717] g Guptasarma P, Balasubramanian D, Matsugo S, Saito I. Biochemistry. 1992; 31:4296. [PubMed: 1567875] h Takeuchi T, Matsugo S, Morimoto K. Carcinogenesis. 1997; 18:2051. [PubMed: 9395201]
6. a Chang C-Y, Hsieh Y-H, Hsieh L-L, Yao K-S, Cheng T-C. J. Hazard. Mat. 2009; 166:897. b Matthews RH. J. Chem. Soc. Chem Comm. 1983:177.
7. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. Cancer Res. 2009; 69:8784. [PubMed: 19887611] Gurr J-R, Wang ASS, Chen C-H, Jan K-Y. Toxicology. 2005; 213:66. [PubMed: 15970370]
8. a Adilakshmi T, Soper SFC, Woodson SA. Methods Enzymol. 2009; 468:239 - 58. [PubMed: 20946773] b Brenowitz M, M. R, C. Dhavan G, Takamoto K. 2002; 12:648-53.
9. Ottinger LM, Tullius TD. J. Am. Chem. Soc. 2000; 122:5901.

10. a Taylor R, Smucker L, Hanna ML, Gill J. Arch. Biochem. Biophys. 1973; 156:521. [PubMed: 4718782] b Schrauzer GN, Lee L-P, Sibert JW. J. Am. Chem. Soc. 1970; 92:2997. [PubMed: 5446952]
11. Shell TA, Mohler DL. Curr. Org. Chem. 2007; 11:1525.
12. Morelli R, Russo-Volpe S, Bruno N, Scalzo RL. J. Agric. Food Chem. 2003; 51:7418. [PubMed: 14640593]
13. Beauchamp C, Fridovich I. J. Biol. Chem. 1970; 245:4641. [PubMed: 5466067]
14. Halliwell B, Gutteridge JMC, Aruoma OI. Anal. Biochem. 1987; 165:215. [PubMed: 3120621]
15. a Gutteridge JMC. FEBS Lett. 1981; 128:343. [PubMed: 7262325] b Cheeseman KH, Beavis A, Esterbauer H. Biochem. J. 1988; 252:64. c Gutteridge JMC, Halliwell B. Biochem. J. 1988; 253:932. [PubMed: 2845941]
16. a Mohler DL, Downs JR, Hurley-Predecki AL, Sallman JR, Gannett PM, Shi X. J. Org. Chem. 2005; 70:909. b Mohler DL, Dain DR, Kerekes AD, Nadler WR, Scott TL. Bioorg. Med. Chem. Lett. 1998; 8:871. [PubMed: 9871557]
17. Connolly TJ, Baldoví MV, Mohtat N, Scaiano JC. Tetrahedron Lett. 1996; 37:4919.
18. Povirk LF, Wübker W, Köhnlein W, Hutchinson F. Nucleic Acids Res. 1977; 4:3573. [PubMed: 73164]
19. Kapp PD. Biochemistry. 1984; 23:4821. [PubMed: 6238622]
20. Lee HM, Larson DR, Lawrence DS. ACS Chem. Biol. 2009; 19:409. [PubMed: 19298086]

Scheme 1.

(A) Photolysis of MeCbl to furnish CH₃· and (B) proposed light-dependent generation of ·OH by B_{12a}.

Figure 1.

Light-driven $\cdot\text{OH}$ production via photolysis of the $\text{B}_{12\text{a}}$ -resin conjugate **2**. The conjugate **2** was suspended in a deoxyribose solution (50 mM in pH 7.4 PBS). The suspension was sequentially photolyzed (60 min total), stored in the dark (100 min total), and photolyzed (60 min total), with aliquots removed and analyzed at various time points.

Figure 2.

Light-mediated DNA (30 μ M/bp in pH 7.4 PBS) by hydroxocobalamin (300 pM). Samples were irradiated with Pyrex-filtered light from a mercury arc lamp.