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Visible Light-Induced Radical Mediated DNA Damage

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Abstract

Light-responsive compounds have been used to manipulate biological systems with spatial and temporal control of the event of interest. Illumination of alkylcobalamins with green light (500 – 570 nm) produces carbon-centered radicals, which have been demonstrated to effectively cause DNA damage. Molecules that cause DNA and RNA strand scission are useful for studying polynucleotide structure and the binding of small molecules and proteins to polynucleotides. Most molecules that cause DNA damage in a light-dependent manner require high energy, short wavelength ultraviolet light, which is readily absorbed by nucleotide bases causing damage to the polynucleotides. Therefore, using alkylcobalamins is advantageous for causing strand scission of polynucleotides, because they are activated by light wavelengths that are not absorbed by nucleotide bases. Green light illumination of methylcobalamin effectively causes DNA strand scission based on gel mobility assays. This cleavage is due to the generation of carbon-centered radicals based on the results of a radical trapping study. In addition, synthesis of an alkylcobalamin with a DNA binding moiety, spermine, improves DNA cleavage efficacy by an order of magnitude in comparison to methylcobalamin.

Graphical abstract

Most molecules that cause DNA damage in a light-dependent manner require high-energy, short wavelength ultraviolet light, which is readily absorbed by nucleotide bases causing damage to polynucleotides. Using alkylcobalamins is advantageous for causing strand scission of polynucleotides, because they are activated by wavelengths not absorbed by nucleotide bases. Using gel mobility assays, we show that green light illumination of methylcobalamin causes DNA strand scission due to generation of carbon-centered radicals, based on the results of a radical trapping study. In addition, synthesis of an alkylcobalamin with a DNA-binding moiety, spermine, improves DNA cleavage efficacy in comparison to methylcobalamin.

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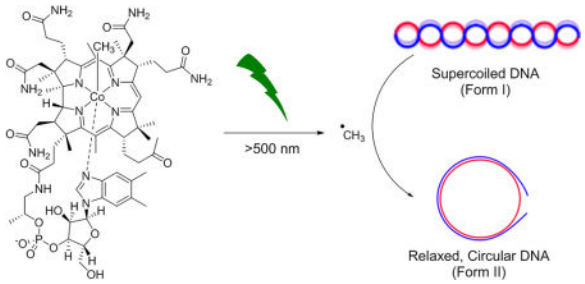

INTRODUCTION

Radicals have been utilized in footprinting studies to determine RNA and DNA structures as well as DNA or RNA-binding sites of proteins and small molecules (1–4). Polynucleotide footprinting studies utilize radicals that cause strand scission to probe for solvent accessible regions of the molecular structure. The most commonly employed means of generating radicals is the Fenton reaction, which produces hydroxyl radicals (•OH) catalytically as a result of the reduction of hydrogen peroxide by Fe(II)-EDTA (5). A limitation of the Fenton reaction is the inability to temporally control the start and stop of radical generation. This drawback causes a challenge with maintaining reaction conditions where each polynucleotide molecule is damaged by only one radical, a necessary condition for footprinting studies. In addition, the lack of temporal control of the reaction prevents its use for intracellular studies.

Photoresponsive compounds afford exquisite temporal control of chemical reactions (6 - 7) For example, several light-responsive reagents have been developed to temporally control radical generation for DNA footprinting (8 - 12). These compounds generate •OH or alkyl radicals, such as methyl (•CH_3) or phenyl radicals, upon illumination with an appropriate wavelength causing DNA/RNA strand scission by abstracting a hydrogen atom from the sugar phosphate backbone of the polynucleotides. The light dependence of these reactions offers temporal control of damage. However, typically a requirement of these compounds is the use of short wavelength, high-energy ultraviolet light (UV, <450 nm), which is readily absorbed by nucleotide bases resulting in a small amount of light-induced polynucleotide damage due to pyrimidine dimerization (13 - 14). This light-induced damage interferes with strand scission patterns due to the radicals complicating the interpretation of footprinting data. Therefore, compounds that generate radicals when illuminated with longer wavelength, visible light (>450 nm), which is not absorbed by nucleotide bases, would be useful footprinting agents.

We have noted the utility of cobalamins (Figure 1), a class of compounds that are structurally related to Vitamin B_{12}, to temporally control activity of compounds and radicals (8, 15–17). For example, we recently reported that when exposed to light thiolatocobalamins undergo homolysis of their cobalt-sulfur bond to form thiyl radicals (18). In addition, we previously demonstrated that hydroxocobalamin (HOCbl) in conjunction with UV light causes DNA strand scission via the production of •OH (8). An advantage of using HOCbl is that in the presence of oxygen it catalytically produces •OH upon illumination, while the majority of light-responsive radical generators are stoichiometric. However, HOCbl, like

other reported light-mediated radical generators, requires UV light that causes a small amount of DNA damage (8).

We were interested in developing light-responsive DNA damaging agents that respond to visible light, which in contrast to UV light does not cause pyrimidine dimers. Therefore, we decided to study the ability of alkylcobalamins to cause DNA strand scission because they absorb green light (510-560 nm) resulting in the production of alkyl radicals via a homolytic cleavage of their cobalt-carbon bond (19–20). Alkyl cobalamins have a cobalt-carbon σ bond that has a relatively weak bond strength. Reported Co-C bond dissociation energies BDEs range from 40-44 kcal/mol depending on the alkylcobalamin and the particular experimental conditions used (21). Photodisocciation pathways of alkylcobalamins depend on both the wavelength and pH. The photochemical properties of alyllcobalamins at low pH, strongly acidic pH (~2) conditions differ greatly from the alkylcobalamins at higher pH because at low pH the alkylcobalamin adopts a base-off form where the dimethylbenzoimidazole (DMB) is not associated as a lower axial ligand. At physiological pH, there are two possible photodissociation pathways (21). The light wavelengths that we were interested in employing for DNA cleavage studies (510-560 nm), result in the initial elongation of the Co-C bond forming metastable cob(III)alamin photoproduct, a low-lying electronic state with a nanosecond lifetime, without prompt bond homolysis being observed (21). Alkyl radicals, such as $\bullet CH_3$ and phenyl radical, have been demonstrated to be effective at causing DNA strand scission (9, 11–12). Therefore, we anticipated that alkylcobalamins would be useful DNA footprinting agents, such as methylcobalamin (MeCbl), would be expected to cause DNA cleavage when illuminated with wavelengths of light that are not capable of causing polynucleotide damage.

We note that photosensitizers that cleave DNA when illuminated with visible light have been reported, such as methylene blue and acridine orange. These agents cause damage by generating reactive oxygen species singlet oxygen and •OH. Singlet oxygen selectively reacts with guanine bases of DNA leading to strand scission (22–23). Polynucleotide footprinting agents need to be nonselective in which bases they react with in order not to distort the data of which areas of the polynucleotide are the most solvent accessible. Methyl radical has been demonstrated to cleave DNA in a nonselective manner (12). Therefore, the use of $\bullet CH_3$ as a footprinting agent is advantageous over systems that generate reactive oxygen species.

MATERIALS AND METHODS

Materials

Plasmid DNA, pBR322, was purchased from New England Biolabs. Methylcobalamin was purchased from Alfa Aesar. *N,N,N′,N′*-Tetramethyl-O-(*N*-succinimidyl)uronium tetrafluoroborate and 4-bromobutyric acid were purchased from Tokyo Chemical Industry. Hydroxocobalamin, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), sodium phosphate monobasic monohydrate, and sodium phosphate dibasic anhydrous were purchased from Sigma Aldrich. Purified, deionized water was obtained by filtration using an Aries High Purity Water System and was used for all aqueous reactions and dilutions. A custom amber glass filter was purchased from Ace Glass, Inc. GelRed nucleic acid stain 10,000× was

purchased from Biotium. Tris-acetate-ethylenediaminetetraacetic acid (TAE) and biotechnology grade agarose were purchased from Amresco. Gel electrophoresis was performed with agarose gels and TAE buffer (40 mM Tris acetate and 1 mM EDTA) in a CBS Scientific Midi horizontal electrophoresis apparatus. The concentrated loading buffer for agarose gels consisted of 35% (w/v) sucrose solution containing 0.2% bromophenol, 0.2% xylene cyanol, and 0.6% GelRed nucleic acid stain 10,000×. Agarose gels were visualized using a VWR transilluminator with UVP PhotoDocIt imaging system.

Synthesis of spermine-cobalamin conjugate - Synthesis of β-(4-carboxybutyryl)cobalamin

β-(4-carboxybutyryl)cobalamin was synthesized using a previously reported procedure (16). Cyanocobalamin and zinc dust (100 equivalents) were mixed in a deoxygenated 5% NH_4Br methanol solution for 20 min. After the reduction of the cobalamin, 4-bromobutyric acid (10 equivalents) was added to the reaction mixture and the solution was mixed for 20 min. Following the completion of the alkylation reaction, the mixture was gravity filtered to remove the zinc dust and was dripped into cold ether resulting in a red suspension. The suspension was centrifuged and the supernatant was discarded. The desired compound was purified by reverse phase flash chromatography (C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% TFA/H_2O; solvent B: 0.l% TFA/CH_3CN) with a ratio of A:B that varied from 97:3 to 50:50 over 10 column volumes. Removal of solvent by lyophilization afforded to afford an orange solid; ESI MS calcd. for $C_{66}H_{95}N_{13}O_{16}PCo$ $[M^+ + 2H]^{2+}$:m/z = 708.8, found 709.0.

Synthesis of spermine-cobalamin conjugate - Synthesis of spermine-cobalamin conjugate

N,*N*,*N*′,*N*′-Tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (0.0115 g, 38.2 μM), β-(4-carboxybutyryl)cobalamin (0.0146 g, 10.3 μM), and diisopropylamine (0.0130 g, 128 μM) were dissolved in dimethylformamide (250 μL), which was mixed for 2 h. Spermine (0.1143 g, 564 μM) dissolved in dimethylformamide (500 μL) was added and the reaction was mixed for 18 hr. The desired compound was purified by HPLC (semipreparative C-18 column) using a linear gradient binary solvent system (solvent A: 0.1% trifluoroacetic acid/H_2O; solvent B: 0.1% trifluoracetic acid/CH_3CN) with a ratio of A:B that varied from 97:3 (0 min) to 10:90 (40 min). Removal of solvent by lyophilization afforded an orange solid (0.0122 g, 74%); ESI MS calcd. for $C_{76}H_{119}CoN_{17}O_{15}P$ $[M + 2H]^{2+}$: *m/z* = 801.9, found 801.5; $[M + 3H]^{3+}$: *m/z* = 534.3, found 535.0.

General procedure for light-mediated DNA cleavage studies

Unless otherwise noted all DNA cleavage studies were performed under aerobic conditions. Methylcobalamin or hydroxocobalamin was dissolved in pH 7.4 phosphate buffer (11.9 mM phosphate) and serial dilutions were made to achieve the desired concentrations. A 2 μL of pBR322 DNA (100 μM/bp) solution were mixed with 18 μL of MeCbl or HOCbl solution of an appropriate concentration in a plastic microcentrifuge tube. Then the tubes were either incubated at room temperature in the dark or were strapped to a water-jacketed reaction vessel with an amber glass filter (>500 nm) and illuminated using a Hanovia 450 W medium pressure Hg arc lamp. After illumination/incubation, 2.0 μL of loading buffer was added and the samples were loaded onto a 1.0% agarose gel. Electrophoresis was performed for 1.5 h at 90 V.

Light-mediated DNA cleavage by methylcobalamin

Samples were prepared to have final MeCbl concentrations of 1.00, 0.750, 0.563, 0.422, 0.316 and 0.237 mM and were illuminated for 45 min.

Methylcobalamin-mediated DNA cleavage as a function of time

Samples were prepared to have a final MeCbl concentration of 1.00 mM. In one time study, the samples were illuminated for 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min. In another time study, the samples were illuminated for 10, 20, 30, 40, 50, and 60 min.

Methylcobalamin-mediated DNA cleavage under anaerobic conditions

These experiments were performed under anaerobic conditions. Samples were prepared to have final MeCbl concentration of 1.00 mM. In one experiment, the anaerobic sample was prepared using a buffer that was deoxygenated by bubbling nitrogen through the solution for 30 min. For another experiment, the degassed sample was prepared by placing it in a Schlenk vial and 5 freeze-pump-thaw cycles were completed prior to illumination. Samples were illuminated for 10 min.

Effect of photolysis of methylcobalamin before the addition of plasmid DNA on the cleavage of DNA

Three samples were prepared, DNA only, DNA and MeCbl stored in the dark and MeCbl that was illuminated for 15 min before the DNA was added. Final concentrations of samples that contained MeCbl was 1 mM.

Radical scavenging of methylcobalamin-mediated DNA cleavage under anaerobic conditions

This experiment was performed under anaerobic conditions. Nitrogen was bubbled through all solutions to displace oxygen before they were used. Due to the low solubility of TEMPO, samples for the radical scavenging experiment were prepared in a different manner than the general procedure for the DNA cleavage studies. TEMPO was dissolved in phosphate buffer. The reaction mixtures were made by combining 0.5 μL MeCbl (20 mM), 0.5 μL pBR322 (400 μM/bp), and 19 μL TEMPO (5.26 mM). Samples were illuminated for 10 min.

Study of the ability of hydroxocobalamin to cause visible light-mediated DNA cleavage

Samples were prepared to have final HOCbl concentrations of 1.00, 0.750, 0.500, and 0.250 mM and were illuminated for 45 min.

DNA cleavage by spermine-cobalamin conjugate

Samples were prepared to have final spermine-cobalamin conjugate concentrations of 0.250, 0.175, 0.122, 0.85, 0.60, 0.42, 0.29, 0.20 and 0.14 mM and were illuminated for 10 minutes.

DNA cleavage by reduced Co(II)-cobalamin, [Co(II)bl]

This experiment was performed under anaerobic conditions. Samples of Co(II)bl were prepared with and without TEMPO. A reducing solution was prepared with 10 μL of 6 M NaOH (108 mM), 3 μL of 88% formic acid, and of 460 μL deionized water. A second

sample was prepared in the same manner except that it contained TEMPO (100 mM). The reaction mixtures were degassed with nitrogen for 20 minutes, then 28 μL of hydroxocobalamin (19.7 mM) was added to each tube and degassed a second time. Final samples were made with 18 μL of the Co(II)bl solution added to 2 μL of DNA for a final concentration of 1 mM cobalamin compounds and 10 μM/bp pBR322 DNA. Samples were incubated in the dark for 5 min. Sodium dodecylsulfate (1 μL, 1% SDS) was added to each tube to prevent DNA precipitation. Samples were analyzed by gel electrophoresis as previously described.

RESULTS AND DISCUSSION

Plasmid DNA cleavage by methylcobalamin

Illumination of MeCbl with visible light results in the formation of reduced Co(II)-cobalamin [Co(II)bl] and methyl radical ($•CH_3$) (19–20), which has been demonstrated to cause DNA strand scission (9, 11–12) Therefore, we were interested in studying the ability of MeCbl to cause DNA cleavage in a visible light dependent manner. To assess the ability of MeCbl to cause DNA cleavage when illuminated with visible light, a plasmid relaxation assay was employed to observe $•CH_3$ mediated conversion of supercoiled DNA (form I) to relaxed, circular DNA (form II). Various concentrations of MeCbl were illuminated with a mercury arc lamp equipped with an amber glass filter (>500 nm) in the presence of pBR322 resulting in single strand cleavage (Figure 2).

DNA strand scission is not observed with MeCbl in the absence of light demonstrating that it is a light mediated reaction. The amount of damage is concentration dependent with no cleavage observed below 56 molecules per base pair. Under our experimental conditions, photolysis of MeCbl occurs rapidly with the maximum DNA strand scission being observed within 5 minutes of illumination. (Figure 3, Lane 8). Most importantly, there is no DNA strand scission due to illumination in the absence of MeCbl. This demonstrates that unlike UV (8,9), the light wavelengths that are employed do not cause any observable DNA cleavage.

A radical trapping study demonstrates the role of $•CH_3$ in causing strand scission under aerobic conditions. The presence of 2,2,6,6-tetramethyl-1-piperdinyloxy (TEMPO), a stable nitroxide that traps carbon-centered radicals, but not oxygen-centered radicals (23), completely prevents DNA cleavage by MeCbl due to TEMPO reacting with $•CH_3$ before hydrogen atom abstraction from the sugar phosphate backbone can occur (Figure 4).

As an additional control experiment to demonstrate the role of $•CH_3$ in DNA strand scission MeCbl was photolyzed and DNA was subsequently added in the dark (Lane 3, Figure 5). Cobalamins are known radical scavengers (25); therefore, generated $•CH_3$ will be scavenged by cobalamin before the addition of the DNA. As expected, no DNA cleavage was observed for this experiment. While some $•CH_3$ are likely scavenged by the cobalamin when MeCbl is illuminated in the presence of DNA, clearly enough $•CH_3$ escape the radical-cobalamin pair to cause DNA strand scission (Lanes 4–5, Figure 2).

The mechanism of DNA damage by •CH_3 has previously been studied (12). Methyl radicals can cause DNA strand scission via two different mechanisms. One mechanism of DNA damage is the direct abstraction of a hydrogen atom from the sugar of the sugar-phosphate backbone (12). The other route that causes cleavage via a methyl radical mediated process involves the radical initially reacting with oxygen to form methylperoxy radicals, which have been suggested to be unable to cleave DNA (26). However, methylperoxy radicals can decompose to form methoxyl radicals (12), which are capable of abstracting a hydrogen atom from DNA leading to strand scission (27). It has been suggested that Co(II)bl and HOCbl are involved in catalyzing the decomposition of alkylperoxy radicals (28). Unfortunately, the mechanisms and products of these cobalamin catalyzed reactions have not been well studied. DNA cleavage mediated by •CH_3 is initiated by hydrogen abstraction at the 4′ and 5′ positions of the sugar as demonstrated by the detection of furfural and base propenals as the products (12). Following hydrogen atom abstraction, the carbon-centered radical of the sugar reacts with molecular oxygen forming an alkylperoxy radical. The details of the reactions that follow depend on the particular hydrogen that was abstracted. In all cases, decomposition of the sugar ring results in causing strand scission.

A hallmark of radical mediated DNA cleavage is the requirement of the presence of oxygen (29). Therefore, anaerobic experiments were performed. Nitrogen gas was bubbled through the solutions for 30 minutes to remove oxygen before they were used to make the experimental solutions. Surprisingly, photolysis of MeCbl causes DNA strand scission in the absence of oxygen (Figures 6a). Due to this unexpected result, we decided to perform the same experiment using a different technique for removing oxygen. Samples underwent five freeze-pump-thaw cycles before photolysis (Figure 6b). The results were consistent with our initial experiment and confirmed that the photolysis of methylcobalamin in the presence of DNA under anaerobic conditions results in strand scission.

Due to the unexpected results from the anaerobic studies, we decided to investigate whether the reduced cobalamin by-product Co(II)bl was capable of cleaving DNA under anaerobic conditions. To prepare Co(II)bl, HOCbl is dissolved in a basic formate solution under anaerobic conditions resulting in the solution rapidly changing color from pink to a reddish brown indicating the formation of Co(II)bl (30). The UV/Vis spectrum of the reaction mixture shows a local maximum absorption at 450 nm that is not observed for HOCbl, which demonstrates the production of Co(II)bl (Figure 7).

Addition of an aliquot of the Co(II)bl solution to a deoxygenated pBR322 solution results in efficient DNA cleavage which is effectively inhibited by TEMPO (Figure 8), a metal-centered radical trap (31).

In addition, photolysis of MeCbl under anaerobic conditions which furnishes Co(II)bl causes DNA strand scission that is prevented by the presence of TEMPO (Figure 9). While these experiments demonstrate the ability of Co(II)bl to cause DNA damage under anaerobic conditions it would not be expected to play a significant role in DNA cleavage by MeCbl because Co(II)bl reacts rapidly with oxygen to form HOCbl (8).

We have previously reported light-mediated DNA cleavage by HOCbl (8). When illuminated with UV light HOCbl catalytically produces hydroxyl radicals based on the results of plasmid relaxation assays and thiobarbituric acid assays. Under the experimental conditions that we previously employed, we demonstrated that UV illumination of MeCbl in the presence of DNA causes strand scission and that it is primarily mediated by •OH due to the observation that cleavage is not significantly decreased by the presence of TEMPO, which does not trap •OH radicals. Under UV illumination MeCbl stoichiometrically produces $•CH_3$ and Co(II)bl that rapidly reacts with oxygen to form HOCbl, which catalytically produces •OH under continued UV illumination. Therefore, DNA damage by MeCbl is primarily due to •OH rather than $•CH_3$ when the sample is exposed to UV light beyond the initial photolysis of MeCbl.

In contrast, our current work demonstrates that cleavage by MeCbl upon illumination with visible light is completely inhibited by TEMPO. This demonstrates that the radicals generated by illumination of MeCbl differ depending on the wavelength of light that is employed. When illuminated with visible light MeCbl undergoes photolysis to generate DNA cleaving $•CH_3$ and Co(II)bl that is subsequently converted to HOCbl. Visible light illumination of HOCbl does not generate •OH; therefore, no DNA cleavage is observed when HOCbl is illuminated with visible light in the presence of pBR322 (Figure 10). Therefore, no additional radicals are generated after the initial $•CH_3$ when MeCbl is illuminated with visible light. This is supported by the observation that increasing visible light exposure beyond 5 min does not increase observed DNA cleavage (Figure 3). This demonstrates that when visible light is employed DNA cleavage is mediated by $•CH_3$ in contrast to UV light-mediated DNA cleavage by MeCbl, which primarily occurs as a result •OH.

This result is supported by a recent report that ultraviolet light (< 350 nm) is required for photoactivation of HOCbl (32,33). Kozolowski and coworkers have done excellent research on photochemical product of •OH by HOCbl (32,33). The results of femtosecond UV-visible transient absorption spectroscopy demonstrate that there at least three electronic states that result from photoexcitation (33). Longer light wavelengths populate the energy minimum corresponding to the pOH/d → σ* electronic state, which is primarily responsible for deactivation. In contrast, shorter light wavelengths populate a repulsive electronic state that is likely dominant pOH/d → σ* or d/pOH → σ* character. This electronic state becomes dissociative at longer Co-OH distances. Therefore, HOCbl generates •OH when exposed to shorter light wavelengths, but not when it is illuminated with longer wavelengths.

Targeting DNA

We have demonstrated with other DNA cleaving agents that conjugation of spermine, a DNA binding polyamine (34), increases the efficacy of the compound (8,35) and that when radicals are generated by these molecules they rapidly react with DNA rather than diffusing away to react with other molecules or DNA bound proteins (35). Therefore, we decided to synthesize an alkylcobalamin-spermine conjugate. Cobalamins have two sites that are readily modified: the β axial position and the 5′-OH of the sugar can both be synthetically manipulated in a facile manner (16) We decided to synthesize an alkylcobalamin conjugate

modified a with spermine moiety at the β axial position (Scheme 1) because upon photolysis the radical that is generated will be held proximal to the DNA due to the radical being covalently connected to spermine.

Synthesis of an alkycobalamin-spermine conjugate (**2**) was readily achieved in two steps. Reduction of cyanocobalamin with zinc dust results in reduced Co(I) cobalamin [Co(I)bl], a species often referred to as a "super nucleophile" that has broad chemical reactivity (36,37). In this case, Co(I)bl undergoes a concerted nucleophilic substitution reaction (S_N2) with 4-bromobutyric acid to form β-(4-carboxybutyryl)cobalamin (**1**). Reaction of **1** with spermine using the coupling agent *N,N,N′,N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) affords **2**. Efficacy of **2** as a DNA cleaving agent is demonstrated by the observation of damage at concentrations as low as 1.4 molecules per base pair (Figure 11), which is an order of magnitude better than MeCbl. This significant increase in DNA cleaving ability in comparison to MeCbl suggests that the alkyl radical is generated near the DNA bound by **2**.

Conclusion

Strand scission of DNA and RNA via radicals has proven useful for studying structure and binding interactions of these biomacromolecules. Photoresponsive compounds that generate radicals are particularly useful for these experiments because they afford temporal control of radical production. The majority of photoresponsive compounds that have been reported for radical mediated polynucleotide scission require high energy, short wavelength UV light. UV light is readily absorbed by nucleotide bases resulting in polynucleotide damage. This damage due to light absorption by nucleotide bases interferes with the diffusible radical based cleavage data that is necessary to determine the structure of polynucleotides and polynucleotide complexes with small molecules or proteins. Plasmid relaxation experiments demonstrate that MeCbl causes DNA strand scission via a mechanism that is dependent on $\bullet CH_3$ when exposed to visible light. Most importantly, cleavage of DNA by MeCbl does not occur in the dark and visible light does not damage DNA in contrast to UV light. Facile modification MeCbl improved the ability of the alkylcobalamin to cause DNA cleavage by an order of magnitude. This demonstrates an ability to add polynucleotide-recognizing moieties, such as those developed by Dervan's group (38), to target DNA/RNA and specific sequences for cleavage.

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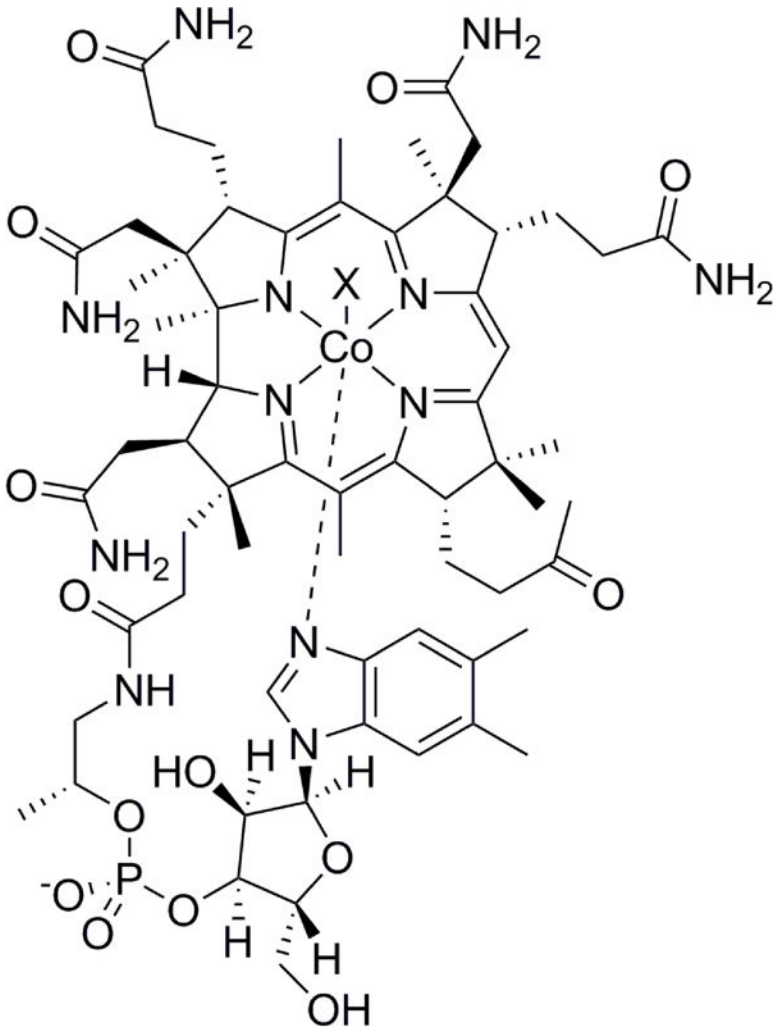


Figure 1.

Structure of methylcobalamin, (X = CH_3), alkycobalamins (X = alkyl group), hydroxocobalamin (X = OH), and thiolatocobalamins (X = SR).

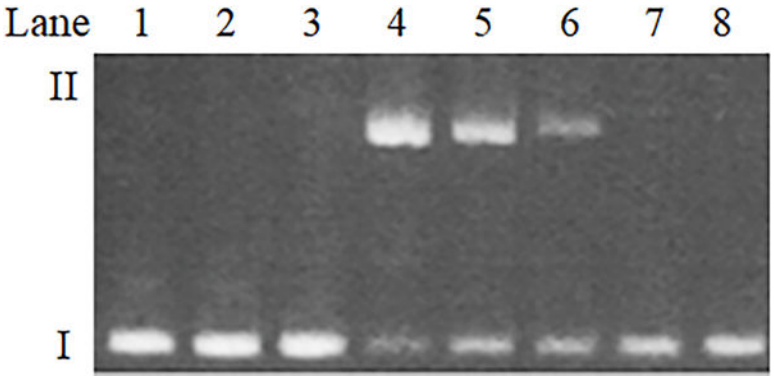


Figure 2.

Visible light-mediated cleavage of pBR322 DNA (10 μM/bp in pH 7.4 phosphate buffer) by methylcobalamin. Lanes 1 and 2, DNA alone; lanes 3 – 8, DNA and methylcobalamin (1000, 1000, 750, 563, 422, and 316 μM, respectively). Samples in lanes 1 and 3 were incubated in the dark, and those in lane 2 and lanes 4 – 8 were illuminated with filtered green light (450 W medium pressure Hg arc lamp) for 45 min.

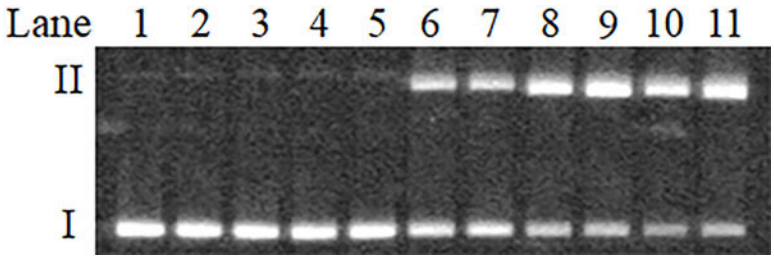


Figure 3.

Visible light-mediated cleavage of DNA by methylcobalamin as a function of photolysis time. Lanes 1 and 2, DNA alone; lanes 3 – 11, DNA (10 μM/bp in pH 7.4 phosphate buffer) and methylcobalamin (1.00 mM). Samples in lanes 1 and 3 were incubated in the dark, and those in lane 2 and lanes 4 – 11 were illuminated with filtered green light (>500 nm, 450 W medium pressure Hg arc lamp) for 8, 1, 2, 3, 4, 5, 6, 7, and 8 min, respectively.

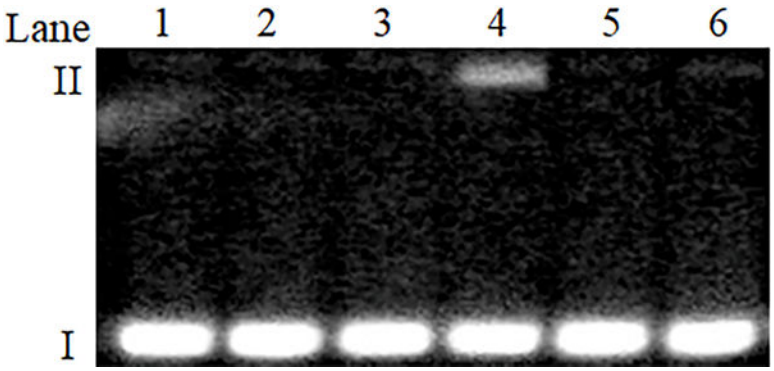


Figure 4.

Radical scavenging of methylcobalamin-mediated DNA cleavage. The effect of TEMPO on the cleavage of pBR322 DNA (10 μM/bp in pH 7.4 PBS) by methylcobalamin was assessed. Lanes 1 and 2, DNA alone; lanes 3 and 4, DNA and methylcobalamin (0.500 mM); lane 5 DNA, methylcobalamin (0.500 mM), and TEMPO (50.0 mM). The samples in lanes 1 and 3 were incubated in the dark, and those in lanes 2 and 4 - 6 were illuminated with filtered green light (>500 nm, 450 W medium pressure Hg arc lamp) for 10 min.

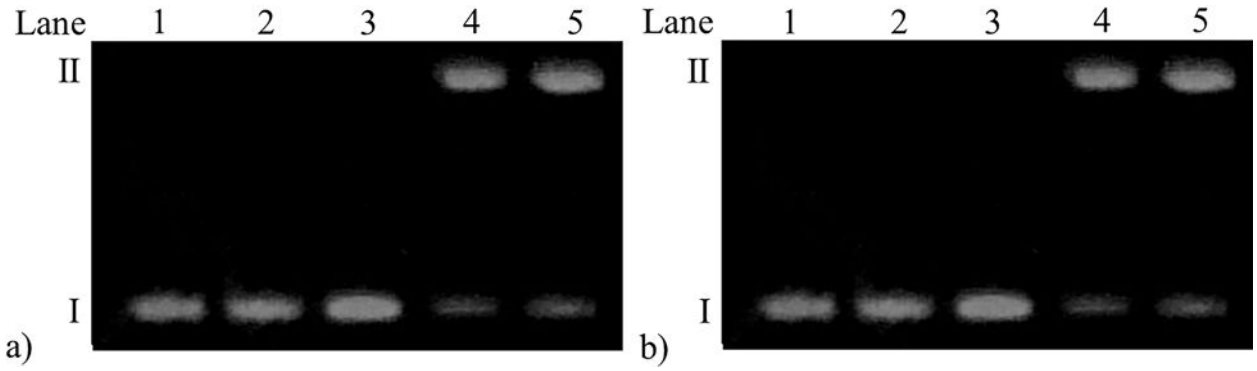


Figure 5.

Effect of photolysis of methylcobalamin before the addition of plasmid DNA on the cleavage of DNA. Methylcobalamin (1 mM) mediated pBR322 DNA (10 μM/bp in pH 7.4 phosphate buffer) cleavage was measured after DNA was added to photolyzed methylcobalamin. Lane 1 pBR322 DNA; lane 2 pBR322 DNA and methylcobalamin; and lane methylcobalamin illuminated with filtered green light (>500 nm, 450 W medium pressure Hg arc lamp) for 15 min before DNA was added in the dark.

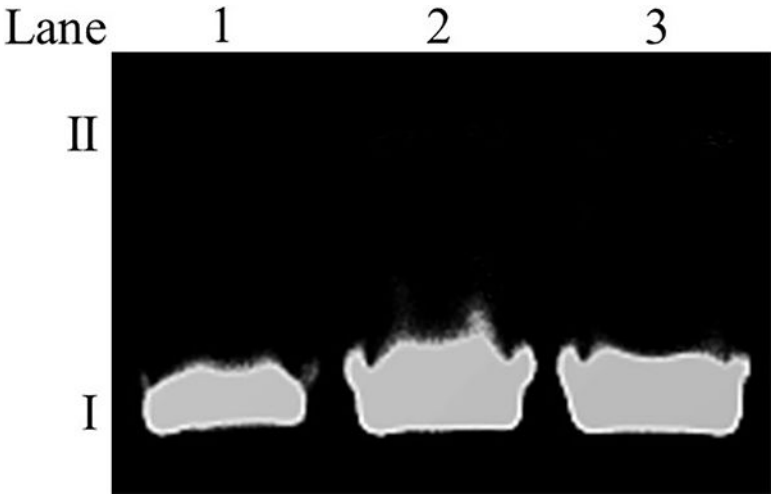


Figure 6.

Visible light-mediated DNA cleavage by methylcobalamin in the absence of oxygen. Methylcobalamin (1 mM) mediated pBR322 DNA (10 μM/bp in pH 7.4 phosphate buffer) cleavage was measured in the presence and absence of oxygen. Lanes 1 and 2, DNA alone; lanes 3, 4 and 5, DNA and methylcobalamin (1.00 mM). a) Nitrogen was bubbled through the buffer of the deoxygenated sample (lane 5) for 30 min. b) The degassed sample (Lane 5) underwent 5 freeze-pump-thaw cycles before being illuminated with light while under vacuum. Samples in lanes 1 and 3 were stored in the dark and samples in lanes 2, 4, and 5 were irradiated with filtered green light (>500 nm, 450 W medium pressure Hg arc lamp) for 10 min.

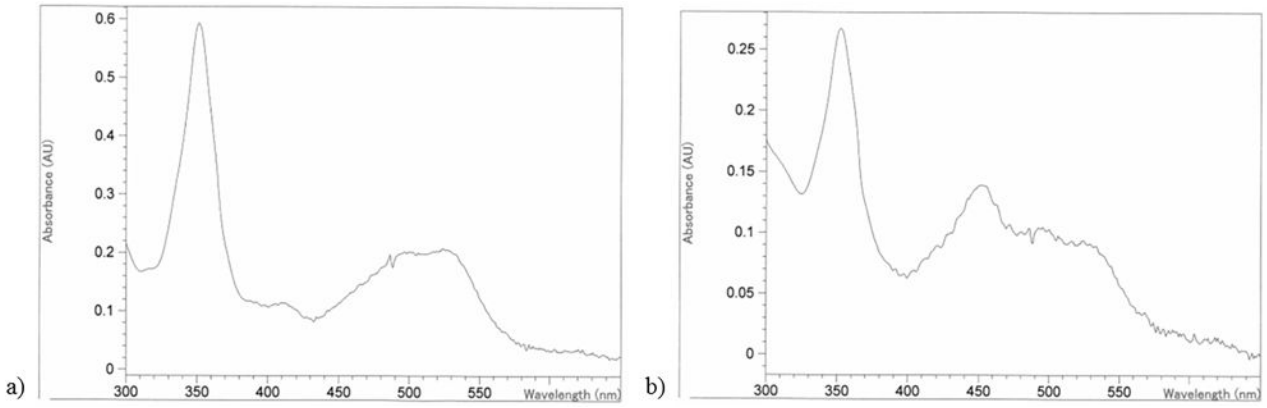


Figure 7.

a) UV/Vis spectrum of hydroxocobalamin. b) UV/Vis spectrum of reduced cobalamain, Co(II)bl.

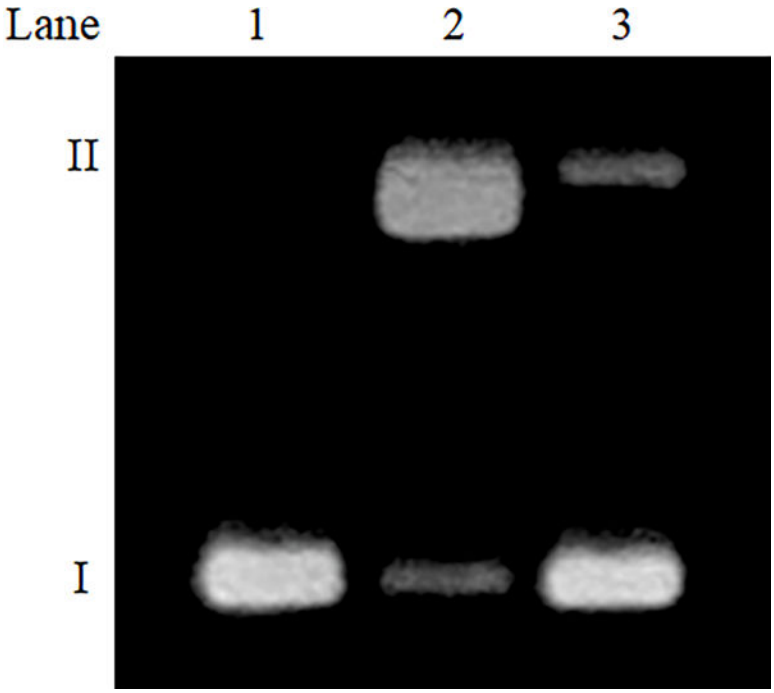


Figure 8.

Anaerobic cleavage of pBR322 DNA (10 μM/bp in pH 7.4 PBS) by vitamin Co(II)bl. Anaerobic DNA cleavage by vitamin Co(II)bl in the presence and absence of TEMPO was assessed. Lane 1, DNA alone; lane 2, DNA and vitamin Co(II)bl (1 mM); lane 3, DNA, vitamin Co(II)bl (1 mM); and TEMPO (50 mM). Co(II)bl was produced by the reduction of hydroxocobalamin by formate. Samples were incubated in dark for 5 min.

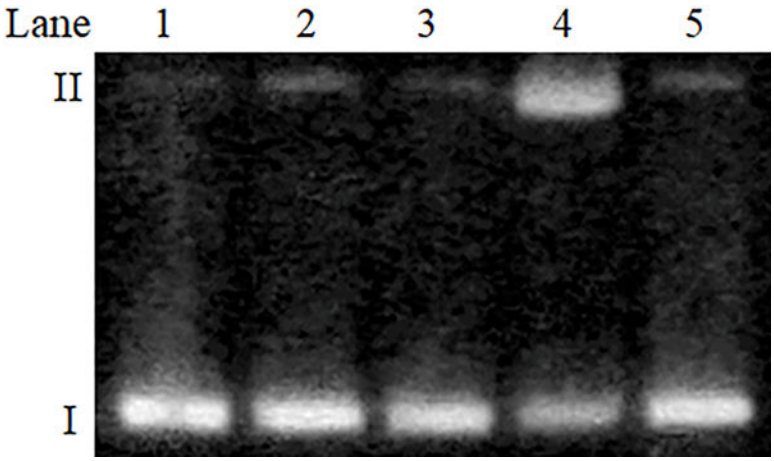


Figure 9.

Anaerobic visible light-mediated DNA cleavage by methylcobalamin in the presence of TEMPO. Methylcobalamin (1 mM) mediated pBR322 DNA (10 μM/bp in pH 7.4 PBS) cleavage was measured in the absence of oxygen. Lanes 1 and 2, DNA alone; lanes 3, and 4 DNA and methylcobalamin; lane 5 DNA, methylcobalamin, and TEMPO (50 mM). Nitrogen on was bubbled through the buffer of the deoxygenated samples (lanes 4 and 5) for 30 min. Samples in lanes 1 and 3 were stored in the dark and samples in lanes 2, 4, and 5 were irradiated with filtered green light (450 W medium pressure Hg arc lamp) for 10 min.

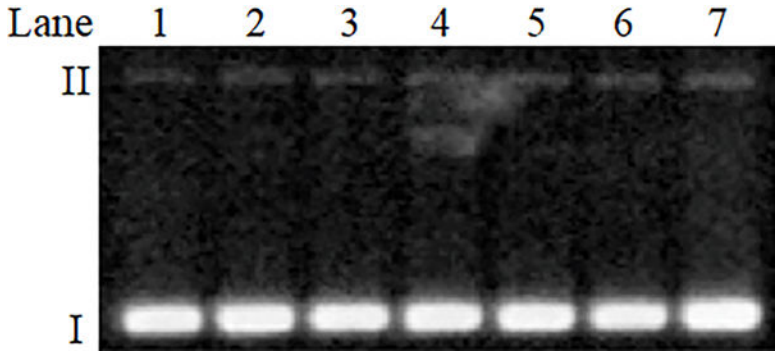


Figure 10.

Hydroxocobalamin is ineffective at causing cleavage of pBR322 DNA (10 μM/bp in pH 7.4 PBS) when illuminated with visible light. Lanes 1 and 2, DNA alone; lanes 3 – 8, DNA and hydroxocobalamin (1.00, 0.750, 0.500, and 0.250 mM, respectively). Samples in lanes 1 and 3 were incubated in the dark, and those in lane 2 and lanes 4 – 8 were illuminated with filtered green light (>500 nm, 450 W medium pressure Hg arc lamp) for 45 min.

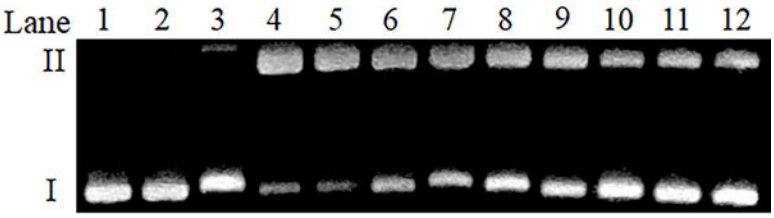
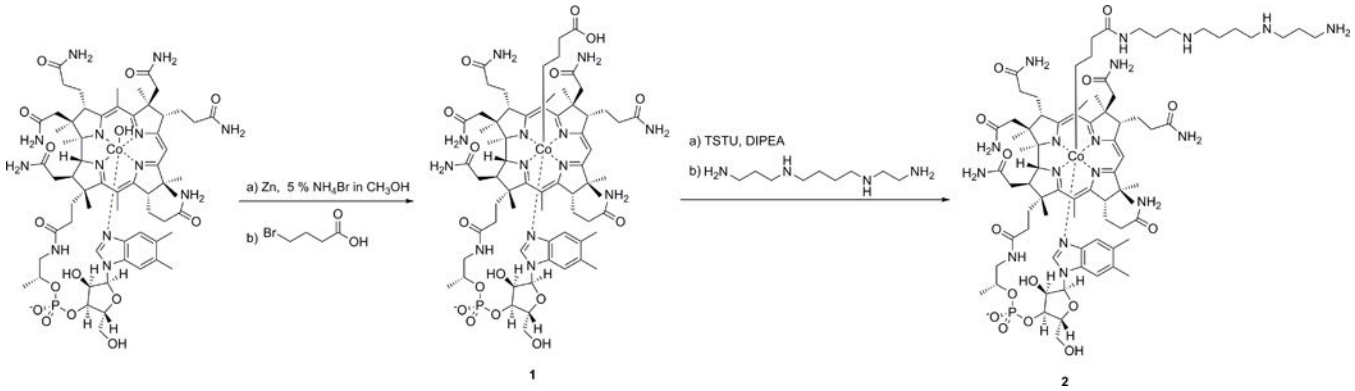


Figure 11.

Visible light-mediated cleavage of pBR322 DNA (10 μM/bp in pH 7.4 phosphate buffer) by spermine-cobalamin conjugate (**2**). Lanes 1 and 2, DNA alone; lanes 3 – 12, DNA and spermine-cobalamin conjugate (250, 250, 175, 122, 85, 60, 42, 29, 20, and 14 μM, respectively). Samples in lanes 1 and 3 were incubated in the dark, and those in lane 2 and lanes 4 – 12 were illuminated with filtered green light (>500 nm, 450 W medium pressure Hg arc lamp) for 10 min.



Scheme 1.
Synthesis of an alkycobalamin-spermine conjugate (**2**).