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Triggering DNAzymes with Light: A Photoactive C8 Thioether-Linked Adenosine

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Light provides an effective means of willfully inducing reactivity to study kinetically complex biological processes and to localize drug action for photodynamic therapy. In most cases, photoactivation is a consequence of irreversible photodeprotection to cleanly unleash the biologically active molecule of interest. Examples include substrates such as ATP,[1a] GABA,[1b] estradiol,[1c] *N*-ras-peptides,[1d] and an mRNA target for ribozymes,[1e,f] allosteric ligands such as cAMP[1g] and inositol triphosphate,[1h] toxins such as ricin,[1i] antibodies to protein-A,[1j] and enzymes such as thrombin[1k,l] and RNaseA.[1m]

Of the many classes of biologically relevant effectors and catalysts, catalytic nucleic acids offer unique properties for controlling gene expression by catalyzing highly efficient M^{2+}-dependent sequence-specific RNA cleavage.[2] To achieve such control, at least two schemes might be envisioned. Covalent attachment of a light-responsive group could (a) constrain the 5′ and 3′ guide sequences to perturb substrate binding[3] or (b) perturb the chemical/conformational nature of the interceding "active site" sequence to impede catalysis. Veiling the active site of a protein enzyme is achieved by covalently attaching a photoactive group (e.g., nitrobenzyl, orthocinnamate) to a nucleophile within the active site, wherein there is often considerable choice with respect to locus and reactivity. In contrast to proteins, the "active site" of an unmodified DNAzyme, absent a 2'OH, affords only the nucleobase amines and carbonyls for covalent protection.

With regard to oligonucleotides, a few reports describe photodeprotection of the exocyclic nucleobase amines protected with a nitroveratrylcarbonyl.[4] Irradiation promotes a cascade of eliminations to yield a nitrosobenzaldehyde and an *N*-carboxynucleoside, which presumably decarboxylates without detection or report.[5] Deprotection yields were variably high upon extended irradiation times: 25−240 min. More rapid deprotection (5−25 min irradiation) was observed with 2,2′-bis(dinitrophenylethoxy)carbonyls. Such deprotection would suggest a useful, yet thus far unexplored, means for unveiling a nucleic acid catalyst.

Our work on modified DNAzymes[6] led us to develop an adenosine analogue, 8-(2-(4-imidazolyl)ethyl-1-thio)-2′-deoxyribo adenosine (d**1**, X = H) and its corresponding phosphoramidite (Figure 1), with an unprecedented photoactivity (λ-max = 279 ± 1 nm) for unveiling DNAzyme activity. Herein, the development of a photoactive DNAzyme exploits the weak carbon−sulfur bond (~68 kcal/mol), which is energetically on par with carbon−halogen bonds and which could be expected to display photoactivity reminiscent of 6-thiopurines[7] and arylthioethers.[8] In contrast to substitution with a monovalent halogen, substitution with divalent sulfur permitted introduction of additional chemical functionality, in this case an ethylimidazole that could perturb the M^{2+} dependence of DNAzymes. The C8 locus was substituted in recognition of (a) the synthetic ease of displacement on the 8-bromo precursor by an alkylsulfide, (b) potential for an altered syn−anti preference around the C1′−N9 purine bond[9] that would perturb DNAzyme conformation, and (c) noted stabilities of both the C8 purinyl radical and anion[10] that might favor either homo- or heterolysis of the C−S thioether to yield adenosine (vide infra).

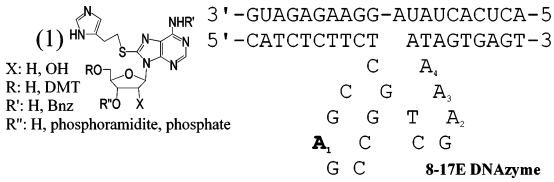

Figure 1. Left: (**1** or d**1**). Right: 8−17E with four singly modified dAs (1−4). Bold A_1 shows inactive substitution. Top: RNA substrate.

The 8−17E DNAzyme,[11] which exhibits robust Zn^{2+}-dependent RNase activity, was chosen to test this strategy; each deoxyriboadenosine was replaced with d**1** to give four variants (8−17E−A_{1-4}) in anticipation that at least one would be completely devoid of catalytic activity.[12] Of these modified variants, A_4 and A_3 remained active, A_2 exhibited 50% reduced activity, and A_1 was utterly inactive (see Supporting Information). This activity profile confirms a recent report that identified A_1 in the stem loop to be essential for activity.[13] Irradiation of 8−17E−A_1 (0.3 μM) in degassed buffer for 8 min with a 10 mW Xe−Hg arc (band-pass filter cutoff > 310 nm) restored ~30% activity of 8−17E (Figure 2). Irradiation with a 254 nm UV lamp (~1 mW) or with a 8 mM dye laser at 283 nm for 10 minutes gave similar results.

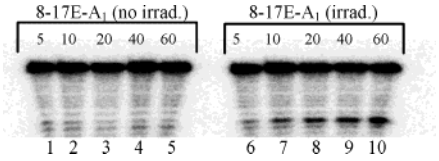

Figure 2. RNA Cleavage activity 8−17E−A_1 before and after 8-min irradiation. RNA cleavage analyzed 5−60 min.

Single turnover kinetics on irradiated 8−17E−A_1 returned a k_{cat} value of 0.113 min^{-1} that was indistinguishable from that of 0.096 min^{-1} obtained for unmodified 8−17E, which was irradiated to control for UV damage (see Supporting Information). MALDI-TOF analysis of 8−17E−A_1 irradiated within the 3-HPA/citrate comatrix indicated that d**1** had converted to dA and not other plausible products of intermediate steric constraint such as 8-oxo-dA or 8-mercapto-dA (see Supporting Information).

To further characterize this reaction in terms of the final purine product, a ribonucleoside **1** (X = OH) was prepared, and its decomposition was characterized by UV−vis, ^{1}H NMR, and ESI spectroscopy. A solution of **1** in degassed CH_3OH/H_2O was irradiated at 280 nm with a 10 mW dye laser. Within 8 min ($t_{1/2} \approx$ 2 min), the λ-max had totally shifted to ~260 nm, a characteristic signature of adenosine (Figure 3). This spectral shift supports the hypothesis that **1** reverted to adenosine and not to 8-oxo- and 8-mercaptoadenosine, which exhibit λ-maxima at 270 and 310 nm, respectively.[14] Indeed, a small shoulder at 310 nm transiently ap-

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peared, suggesting the possibility of a discrete intermediate. Following irradiation in CH_3OH, ESI analysis of the crude gave one peak MH^+ of A: found/calculated 268.1 (Supporting Information), which substantiated reversion to adenosine as initially hypothesized. ^1H NMR also indicated a reversion to A (Supporting Information). This degradation raises mechanistic questions regarding C−S bond cleavage.

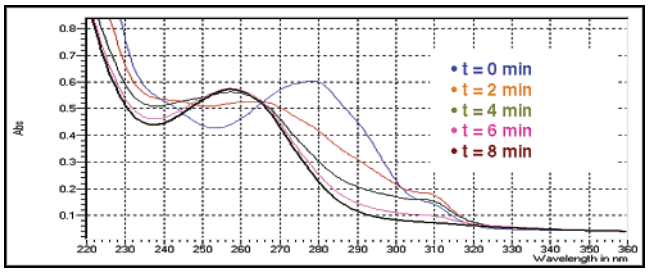
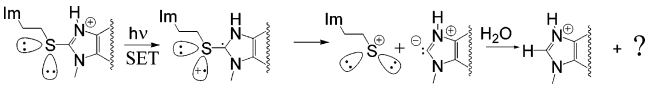


Figure 3. Time course of UV irradiation at 280 nm of 40 μM **1**.

Whereas both homolysis and heterolysis have been proposed for arylthioether fission, homolysis generally predominates.[7] With no precedent for *this* thiopurine fragmentation and only scant data in general for thioether photolyses, we considered both homo- and heterolysis. Homolysis would generate a C8 radical that would abstract an H-atom from a suitable carbon, of which the likely candidate would be a departing imidazolylethylthiyl radical. Heterolysis would normally generate a sulfide and a C8 carbocation that would quench to give 8-oxoadenosine (unobserved). Heterolysis with inverse electronic demand or photoinduced SET into the purine[15a,b] followed by C−S homolysis would ultimately yield an unstable sulfenic acid and a σ-anion at C8 that would protonate in water to give adenosine. N7 protonation could stabilize this anion as an ylid or as a carbene (Scheme 1).

Scheme 1. Photoinduced SET Followed by Homolysis of the C−S Bond[a]



[a] Shown is the carbanion form. Alkyl fragment is unknown.

Since each mechanism stipulates different sources of hydrogen at C8, deuterium labeling would differentiate the two. A quantity of 25 nmol **1** (40 μM) was irradiated in either 1:1 D_2O/CD_3OD or *pure* D_2O, diluted in CH_3OH, lyophilized, and resuspended in 1 mL of CH_3OH to protonate the exchange-labile ND_2 and 2′,3′,5′-ODs. In both cases, ESI^+ mode gave a single peak (MH^+ 269.1118 ± 0.1) with negligible values at 270 or higher, indicating stable monodeuteration. Following irradiation of **1** in CD_3OD, ^1H NMR showed loss of H8 (Supporting Information). H/D exchange on C8 under ambient conditions and upon photoexcitation is negligible and cannot explain these data.[16] As H8 derives from water (or solvent) and not carbon, an intermediate C8 anion is favored over a C8 radical, which would otherwise have to abstract a hydrogen atom from water giving a high-energy hydroxyl radical.

In conclusion, the salient issues in this communication include the disclosure of a photoactive alkylthioadenosine analogue that undergoes rapid and high-yielding cleavage, resulting in reversion to adenosine by what appears to be a novel mechanism. 8-Thioether-linked purines have been but minimally explored, and this report is the first to define the photochemical properties thereof.[17] Other thioether derivatives, including 8-ethylthio-adenosine, were equally reactive, indicating that the imidazole was not responsible for this photoactivity (data not shown) and will be described in a future study that should identify the fugitive alkylthio fragment and full mechanism of fragmentation. The application of this chromophore is highlighted by the generation of a DNAzyme that is photoactivated over a period of minutes at relatively low wattage compared to most photodeprotection procedures. The ease at which this anion is generated may find practical synthetic utility. Furthermore, this study suggests a "post-selection" synthetic strategy for conferring photoactivity on other DNAzymes that should be readily applicable to ribozymes where **1** may be incorporated to distinguish slow folding from fast cleavage. In addition, d**1** may find utility in generating an anion on DNA for electron transport studies. Phosphates of **1** may find use in the study of adenosine processing enzymes (e.g., kinases, polymerases, cAMP-diesterases). Finally, the purine may be used in "capture and release" linkers for proteomics.[18]

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Supporting Information Available: Synthetic and kinetic protocols and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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