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Photochemical DNA Activation

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ABSTRACT



A new photocaged nucleoside was synthesized and incorporated into DNA with the use of standard synthesis conditions. This approach enabled the disruption of specific H-bonds and allowed for the analysis of their contribution to the activity of a DNAzyme. Brief irradiation with nonphotodamaging UV light led to rapid decaging and almost quantitative restoration of DNAzyme activity. The developed strategy has the potential to find widespread application in the light-induced regulation of oligonucleotide function.

Many recent discoveries have revealed the multifactorial roles oligonucleotides play in vitro and in vivo. It has been demonstrated that they can act as catalysts (ribozymes and DNAzymes),[1,2] sensors (aptamers),[3] gene expression platforms (riboswitches and antiswitches),[4,5] and gene regulatory elements (antisense DNA, siRNA, and miRNA).[4,6]

To study the function of oligonucleotides in a detailed fashion and to employ them as highly specific biological research tools, precise control over their activity in a spatial and a temporal manner is required. In this context, light represents an ideal control element since it can be precisely controlled in amplitude, location, and timing thus imposing spatio-temporal control on the system under study.[7] The most common technique of conveying light-regulation to biological processes involves the installation of a photoprotecting group on a biologically active molecule that can be completely removed via light irradiation. This process, termed "caging", has been successfully employed to the light-controlled activation of small molecule inducers of gene expression, fluorophores, peptides, and proteins.[7] DNA and RNA have been caged as well, mostly through statistical reaction of the phosphate backbone of the synthesized or transcribed oligonucleotide with reactive diazo-derivatives of caging groups.[8] The major disadvantage of this approach is that no control over the position and number of installed caging groups can be achieved. Moreover, caging groups are only installed on the phosphate backbone, not on the heterocyclic base itself, failing to disrupt Watson−Crick base pairing. Recently, approaches to the site-specific caging of DNA have been reported. The introduction of an O-4 caged thymidine has been successfully applied to the photochemical activation of transcription and aptamer binding.[9] However, due to the lability of the caging group special DNA synthesis conditions were necessary. An adenosine modified with a sterically demanding, photoremovable imidazolylethylthio group has been used to photochemically activate an 8−17E DNAzyme.[10] After irradiation for 8−10 min with short-wavelength UV

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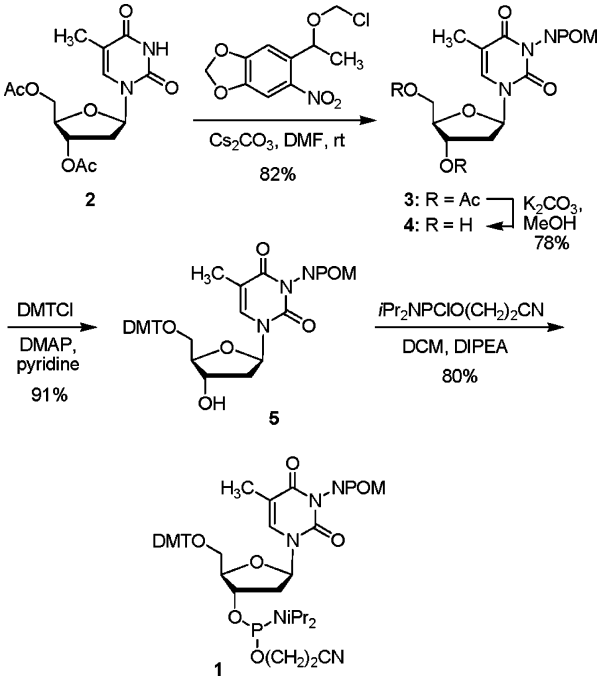
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light (254−310 nm) only 30% of RNA cleavage was observed after a 60 min reaction time. Since the caging group was installed at C-8, no hydrogen bonding of the adenosine was disrupted. Reversible switching of DNAzyme activity was previously achieved through incorporation of diazobenzene motifs; however, only a 5- to 9-fold rate modulation upon irradiation was obtained.[11]

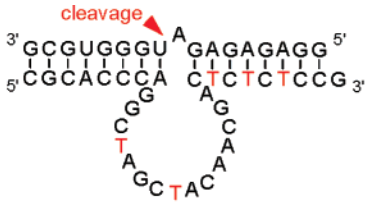
Our goal was to develop a caging approach that fulfills all of the following requirements: (a) allows for specific probing of hydrogen bonding of oligonucleotide bases, (b) enables introduction of the caged monomer under standard DNA synthesis conditions, (c) provides a caged oligomer that is stable to a wide range of chemical and physiological conditions, and (d) allows for excellent restoration of DNA activity upon brief irradiation with nonphotodamaging UV light. Recently, we developed a new caging group (NPOM = 6-nitropiperonyloxymethyl) that proved to be highly efficient in the caging of nitrogen heterocycles.[12] This group was specifically designed to solve previous problems associated with chemical stability or slow decaging rates of photoprotecting groups on nitrogen atoms. Here, we report the application of this group to the caging of the thymidine N-3, thus disrupting an essential hydrogen bond. The phosphoramidite **1** (Scheme 1) was synthesized in 5 steps

Scheme 1. Synthesis of the Caged Phosphoramidite **1**



from thymidine starting with the preparation of the known acetylated thymidine **2** (Ac_2O, DMAP, 98%).[13] Caging with 6-nitropiperonyloxymethyl chloride (NPOM-Cl)[12] was achieved in 82% yield (Cs_2CO_3, DMF, rt) furnishing **3**. Removal of the actetate groups (K_2CO_3, MeOH, 78%) toward **4** followed by selective tritylation of the primary hydroxy group (DMTCl, DMAP, pyridine) delivered **5** in 91% yield. Installation of the phosphoramidite (2-(cyanoethyl)diisopropylchlorophosphoramidite, DCM, DIPEA) was achieved in 80% yield under classical conditions completing the synthesis of **1**.

The stability of **1** to DNA synthesis conditions and its rapid decaging through irradiation with UV light of 356 nm (ϵ_{365} = 6887 cm^{-1} M^{-1}) was demonstrated (see the Supporting Information). The quantum yield (ϕ = 0.094) for the photochemical removal of the NPOM group was determined by 3,4-dimethoxynitrobenzene actinometry.[14] With the use of standard DNA synthesis conditions **1** has been incorporated at all thymidine positions of the 10−23 DNAzyme **D1** providing the mutants **D2**−**D7** (Figure 1). The 10−23



D1 5' CGCACCCAGGCTAGCTACAACGACTCTCTCCG 3'
D2 5' CGCACCCAGGCT_{12}AGCTACAACGACTCTCTCCG 3'
D3 5' CGCACCCAGGCTAGCT_{16}ACAACGACTCTCTCCG 3'
D4 5' CGCACCCAGGCTAGCTACAACGACT_{25}CTCTCCG 3'
D5 5' CGCACCCAGGCTAGCTACAACGACTCT_{27}CTCCG 3'
D6 5' CGCACCCAGGCTAGCTACAACGACTCTCT_{29}CCG 3'
D7 5' CGCACCCAGGCTAGCTACAACGACT_{25}CT_{27}CT_{29}CCG 3'

Figure 1. 10−23 DNAzyme bound to its RNA substrate; thymidines are highlighted in red. Wild-type DNAzyme **D1** and DNAzymes **D2**−**D7** having **1** incorporated at various thymidine positions.

DNAzyme is a highly active and sequence-specific RNA cleaving deoxyoligonucleotide.[2,15] It has been successfully applied to the suppression of genes in vitro and in model organisms.[16]

To probe the necessity of free 3-NH groups in these thymidine residues for the maintenance of DNAzyme activity, the RNA substrate 5′-GGAGAGAGAUGGGUGCG-3′ was radioactively 5′-labeled with ^{32}P-ATP and exposed to the seven DNAzymes **D1**−**D7** in a standard reaction buffer (100 mM $MgCl_2$, pH 8.2, 15 mM Tris buffer) for 30 min at 37 °C (Figure 2). As expected, the original 10−23 DNAzyme **D1** led to almost complete RNA cleavage. DNAzyme **D2** exhibited completely inhibited activity due to the installation

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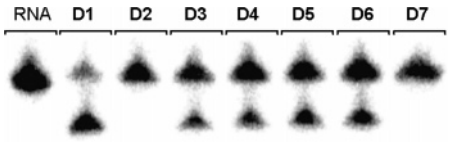


Figure 2. Cleavage of the RNA substrate for 30 min with the 10−23 DNAzymes **D1**−**D7** without prior UV irradiation. Conditions: 100 mM $MgCl_2$, pH 8.2, 15 mM Tris buffer, 37 °C, 40 nM substrate, 400 nM enzyme.

of a single caging group on T_{12}. This was expected, since a previous mutagenesis study of the catalytic core revealed this to be an essential residue.[17] These experiments also demonstrated that the least essential thymidine residue is located at position 16. This was confirmed through the incorporation of **1** at this position leading to still catalytically active **D3**, even in presence of the sterically demanding caging group. We then probed the tolerance of base pair mismatches in the substrate recognition domains by caging the thymidine residues T_{25}, T_{27}, and T_{29}. The resulting DNAzymes **D4**−**D6** displayed lower activity but still induced substantial RNA cleavage. Previously, single mismatches between the RNA substrate and the flanking regions have led to reduced cleavage activity as well.[15] However, selective installation of three caging groups on T_{25}, T_{27}, and T_{29} leads to complete inhibition of RNA cleavage activity in **D7**, presumably due to the disruption of multiple Watson−Crick base paring interactions with the substrate.

Subsequently, a more detailed time-course investigation of the light-activation of **D2** was conducted (Figure 3). A

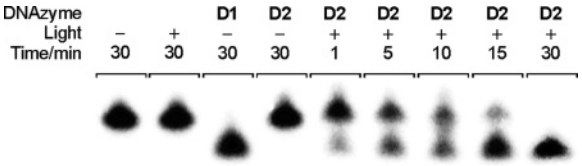


Figure 3. Progressing cleavage of the RNA substrate with the 10−23 DNAzyme **D2** after a 1 min UV irradiation (365 nm). Complete RNA cleavage is observed after 30 min. Conditions: 100 mM $MgCl_2$, pH 8.2, 15 mM Tris buffer, 37 °C, 40 nM substrate, 400 nM enzyme.

control experiment of just the RNA substrate exposed to UV light did not result in any cleaved product. Complete cleavage of the RNA substrate was achieved within 30 min with use of the unmodified **D1**, whereas no cleavage was observed with caged **D2** under identical conditions. However, brief irradiation with nonphotodamaging UV light of 365 nm (25 W) for 1 min initiated decaging and activation of **D2**. Figure 3 displays the resulting RNA cleavage with complete consumption of the substrate by 30 min.

To determine the cleavage rates k of the DNAzymes **D1**, **D2**, and **D7**, the amount of cleaved RNA was quantified at nine different time points under single-turnover conditions through integration (using Molecular Dynamics ImageQuant 5.2) of the corresponding radioactive bands in 15% denaturing TBE polyacrylamide gels with a PhosphorImager (Figure 4).

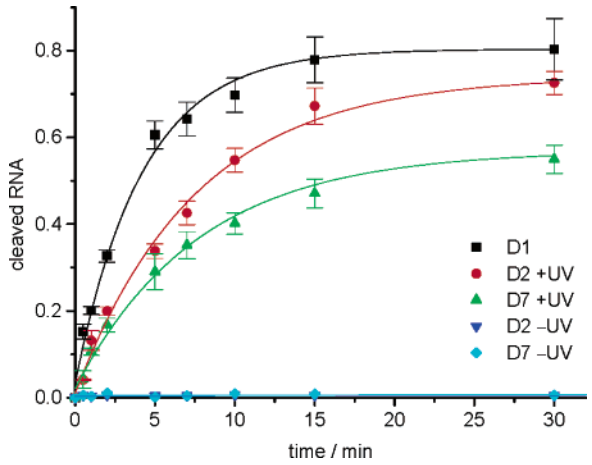


Figure 4. Cleavage of the RNA substrate with the wild-type DNAzyme **D1** and the caged DNAzymes **D2** and **D7** (with and without UV irradiation). Conditions: 10 mM $MgCl_2$, pH 7.4, 15 mM Tris buffer, 37 °C, 40 nM substrate, 400 nM enzyme. The cleaved RNA has been normalized and the experiments were conducted in triplicate.

The data were fitted (using Microcal Origin 5.0) with an exponential decay curve of $-e^{kt}$,[18] and as previously observed, the wild-type DNAzyme **D1** showed a high cleavage rate ($k_{D1} = 0.242 \pm 0.013$ min^{-1}) under the assay conditions. As expected from the results shown in Figure 2, the caged DNAzymes **D2** and **D7** displayed no cleavage activity (k_{D2} = ND and k_{D7} = ND), demonstrating that caging group installation on thymidine can completely abrogate both catalytic activity and substrate binding. Gratifyingly, brief irradiation for 1 min (365 nm, 25 W) of the caged DNAzymes led to restoration of catalytic acitivity of **D2** ($k_{D2,UV} = 0.131 \pm 0.007$ min^{-1}) and **D7** ($k_{D7,UV} = 0.129 \pm 0.011$ min^{-1}) to 54% and 53% of the original **D1** activity, respectively. After a 30 min incubation time 80% of the RNA substrate was cleaved by **D1**, 73% by irradiated **D2**, and 55% by irradiated **D7**. Thus DNAzymes with an excellent light-triggered switch have been developed.

In summary, we synthesized a new photocaged nucleoside, which was incorporated into DNA by using standard synthesis conditions. This caging approach was then used to probe the necessity of specific hydrogen bonds for activity of a DNAzyme, and we found that disruption of a single H-bond can be sufficient to completely inhibit the enzyme. Surprisingly, installation of the bulky caging group was

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tolerated at several positions within the DNAzyme and the caging of three thymidine residues was necessary to abrogate binding to the RNA substrate. Restoration of DNAzyme activity was achieved through decaging with a brief irradiation of 365 nm UV light (UVA light of this wavelength is far less toxic to cells than UVB light of shorter wavelength and is typically considered to be nonphotodamaging[7c,19]), providing an excellent on/off switch for oligonucleotide activity.

We believe that the photocaged phosphoramidite **1** will find widespread application in the light-induced regulation of oligonucleotide function. Most importantly, caged DNAzymes could allow for the spatio-temporal control of gene suppression in model organisms, thus providing powerful tools for functional genomics studies.

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Supporting Information Available: Synthesis and analytical data of **1**−**5** and **D1**−**D7**, enzyme assay protocols, and enzyme decaging study. This material is available free of charge via the Internet at http://pubs.acs.org.

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