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Control of DNA Hybridization with Photocleavable Adducts¶

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ABSTRACT

Previous reports have shown that 1-(4,5-dimethoxy-2-nitrophenyl)ethyl ester (DMNPE) adducts coupled to DNA plasmids block transcription *in vitro* and *in vivo* until removed with light. In this report, we explore the use of DMNPE to control DNA hybridization. We found that DMNPE-caged oligonucleotides have changed spectrophotometric and electrophoretic properties that can be restored with light exposure. Caged oligonucleotides have slower electrophoretic mobility than noncaged oligonucleotides and caged oligonucleotides exposed to light. Effects of caging on hybridization were assessed in a fluorescence-based assay using a 20mer caged DNA oligonucleotide complementary to a 30mer molecular beacon. Fluorescence results indicate that hybridization is reduced and subsequently restored by light. Subsequent gel shift assays confirmed these results. Hybridization activity of caged oligonucleotides with an average of 14–16 DMNPE adducts per oligonucleotide was 14% of noncaged control oligonucleotides and after 365 nm photolysis, increased to nearly 80% of controls. Spectrophotometric characterization of caged oligonucleotides exposed to light and then filtered to remove the released DMNPE adducts indicates two to four attached cage groups remaining following photoactivation. These results suggest that this light-based technology can be used as a tool for the spatial and temporal regulation of hybridization-based DNA bioactivity.

INTRODUCTION

The hybridization of nucleic acids with their complementary strands is a rate-limiting step in many biological processes and bioassays including fluorescence *in situ* hybridization (FISH), microarrays, polymerase chain reaction (PCR), DNA-based biosensors, molecular computing, RNAi and nanomachines. Strategies to enhance, limit or trigger these biological processes often target the hybridization event. It is well known that the extent of DNA hybridization is significantly dependent on temperature and ionic environment, and changes in these have therefore been used to control hybridization. Examples of temperature control are hot-start methods commonly used in PCR. Ionic environmental control of hybridization has been demonstrated through environmental modulation of salts or metal ions (1). A recent work accomplishes spatial and temporal control through inductive coupling of oligonucleotides to metal nanocrystals that provide local heating when exposed to an external radio frequency field (2). Here we describe a strategy to control hybridization of nucleic acids with light, which also enables precise regulation at the onset. Light in the 365 nm range was used to activate oligodeoxynucleotides (ODNs) that were previously inactivated with 1-(4,5-dimethoxy-2-nitrophenyl)diazoethane (DMNPE), a photocleavable ''cage'' compound that has been shown to control bioactivity of DNA plasmids (3).

Caged (i.e., photocaged) compounds have a covalently attached group that can be photocleaved when exposed to specific wavelengths of light. The term *caged* describes the blockade of biological activity rather than a chemical structure (4,5). Examples of studies that have shown control with caged compounds include transport of proteins, and effects on biological membranes as well as muscle fibers (6–9). Cage compounds have been used to study the time course of fast biological processes induced by a millisecond step increase in the intracellular concentration of a bioactive compound of interest achieved by a pulse of light exposure. In many of these applications, the advantages of using photoprotection over conventional methods for substrate release are that the caged effectors can be dispersed throughout the biological target without eliciting the species normal bioactivity. The concentration and spatial distribution of the effector can be controlled, and the temporal release can be varied from seconds to nanoseconds (10).

Compounds that can be light-activated have been used to modulate many aspects of DNA chemistry and biology. Caged compounds have been used in the caging of nucleotide analogs (11), in the synthesis of biochip oligonucleotides (12), in studies of the kinetics of DNA repair (13–15) and as protecting groups during DNA synthesis (16). Cage groups have also been shown to control an oligonucleotide hairpin configuration and, indirectly, hybridization (17). The use of cage compounds has also been observed in the repair of DNA that has a single-strand break at a specific site (18). Inclusion of caged adenosine within synthesized RNA oligonucleotides enables temporal control of ribozyme and spliceosome activities (19,20).

We have also shown that direct caging of plasmid DNA with DMNPE can block transcription, allowing for the targeted

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Abbreviations: DMNPE, 1-(4,5-dimethoxy-2-nitrophenyl)ethyl ester; ODN, oligodeoxynucleotide.
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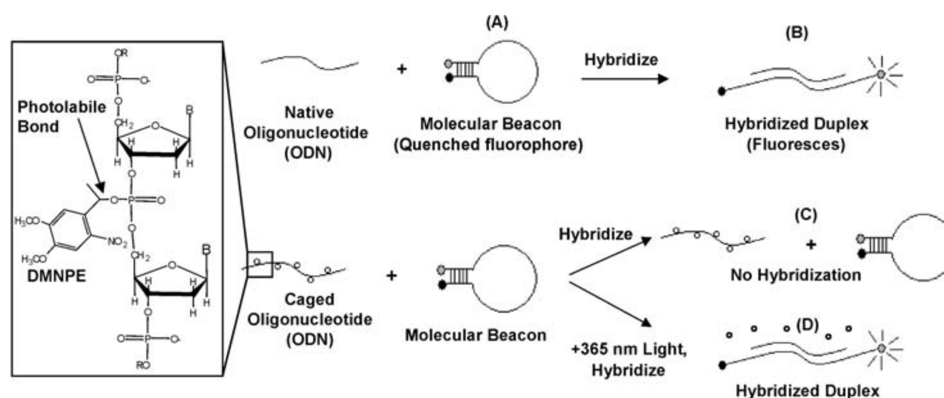

Figure 1. Hybridization assay of caged DNA oligonucleotide (ODN) and complementary molecular beacon. Nonhybridized molecular beacon assumes the stem-loop configuration (A) and does not show fluorescence. When hybridized to a complementary ODN, the beacon fluoresces (B). Caged ODN does not hybridize with molecular beacon (C) until photoactivated with 365 nm light (D). Inset shows theoretical structure of DMNPE-caged ODN with photolabile attachment at the DNA phosphate backbone.

expression of transgenes *in vitro* and *in vivo* through direct light exposure (3). In addition to controlling the kinetics of a particular molecular target, caging affords the ability to restrict reactivation to a localized tissue of interest, as demonstrated by the targeting of transgene expression by caging hormone inducers or nuclear receptor agonists (21–23). More recently, caged mRNA was microinjected in zebrafish embryos to induce expression of certain genes and study the effect of their expression on developmental patterns (24,25). In this report, we explore the application of cage compounds that can be photoactivated to reversibly block hybridization of DNA oligonucleotides.

MATERIALS AND METHODS

ODN caging with DMNPE. Unless otherwise specified, reagents were purchased from Sigma-Aldrich (St. Louis, MO). 1-(4,5-Dimethoxy)-2-nitroacetophenone hydrazone (5 mg, Molecular Probes, Eugene, OR) and manganese (IV) oxide (50 mg) were gently agitated in 1 mL of *N,N*-dimethyl formamide (DMF) at 25°C for 20 min. Manganese oxide was removed from the DMNPE by filtering the solution through Celite (100 mg) supported by glass wool in a 1 cc tuberculin syringe barrel. The filtrate (100 μL) was agitated with the ODN (100 μg, 2 μg/μL) in 10 m*M* Bis-Tris (200 μL, pH 5.5) for 1 h at 4°C. Another 60 μL of the active DMNPE (filtrate) was then added and the solution was agitated for 24 h at 4°C. To remove excess unattached cage compound, caged ODNs were purified using Microcon YM-3 (3000 MW cutoff) centrifugal filters (Millipore, Bilerica, MA). Caged ODNs were spun at 12 110 **g** for 100 min, and then re-suspended in 33% DMF, stored at 4°C and protected from light.

Spectral scanning protocol and DMNPE extinction coefficient determination. Absorption spectrophotometry of caged species was used to estimate the degree of caging. Native (noncaged) 20mer ODN (GCCCAAGCTGGCATCCGTCA, purchased from Integrated DNA Technologies, Coralville, IA) and DMNPE-caged ODN were dissolved in 33% DMF in separate cuvettes (DMNPE-caged ODN, 50 μg/mL; native ODN, 50 μg/mL) and scanned for absorbance from 230 to 500 nm (Thermo Spectronic Genesys 6, Waltham, MA). Spectral scans of caged-flashed ODNs (caged ODNS exposed to light) were also made similarly to the methods described above. To determine the spectral changes following photoactivation, some of the caged-flashed ODN products were purified with the Microcon YM-3 filters following light exposure to remove the released 1-(4,5-dimethoxy-2-nitrosophenyl)-ethanone, and then scanned as described. These samples are referred to as purified caged-flashed ODNs.

To approximate the extinction coefficient of DMNPE adducted ODNs, absorbance peaks at 260 and 355 nm of known concentrations of dilutions of DMNPE-caged ATP in 33% DMF were scanned as described above. The amount of DMNPE present was calculated and the amount of 260 nm absorbance attributed to the DMNPE cage molecules in the spectra of caged ODNs was determined.

Photoactivation and gel electrophoresis of caged ODNs. ODN (250 ng) was run in a 15% polyacrylamide-urea denaturing gel in tris-borate (TBE) buffer (100 m*M* tris-borate, 2 m*M* EDTA pH 8.5) at 70 V for 80 min. Caged-flashed samples were prepared by taking aliquots from the 50 μg/mL caged-ODN samples and exposing them to 365 nm light (dose equivalent to 5.6 J/cm^2) for 20 min before electrophoresis or use in other assays. The light source has a peak output at 365 nm and a fluence rate of 4.68 mW/cm^2 at 10 cm (Model B 100 AP; UVP Blak Ray; San Gabriel, CA). Spectrographic characterization of this lamp confirmed that the emission spectrum is 365 nm ± 8 nm (USB2000 Fiber Optic Spectrometer; Ocean Optics, Inc., Dunedin, FL). Gels were stained after electrophoresis with 1× SYBR-Gold nucleic acid gel stain (Molecular Probes) in TBE buffer for 30 min.

Hybridization of ODNs to complementary molecular beacons and ODNs. To determine the effects of DMNPE attachment on DNA hybridization activity, an assay using molecular beacons was developed. Molecular beacons are fluorogenic probes that signal hybridization with a complementary nucleic acid target (26). These DNA oligonucleotides contain a 5′ fluorophore, a 3′ quenching group, and 4–6 complementary bases on the 3′ and 5′ stem ends, which cause the beacon to form a hairpin structure. Unless the inner loop region hybridizes to a complementary nucleic acid, the fluorescence of the beacon in its hairpin configuration is quenched (Fig. 1). When hybridized with a complementary ODN, the hairpin structure linearizes, distancing the fluorophore from the quencher generating fluorescence (27).

A molecular beacon (MB1) was designed to hybridize with the specified ODN used in this experiment (28). The sequence of MB1 was designed with the aid of *mfold,* an RNA/DNA folding analysis program (29) and synthesized by Biosearch Technologies (Novato, CA). The sequence of MB1 is 5′-FAM-gtgcgTGACGGATGCCAGCTTGGGCcgcac-BHQ1-3′, where capital letters indicate bases complementary to the caged and native ODN, and lowercase letters indicate bases forming the stem region of the beacon. The quenching group in this beacon is BlackHoleQuencher-1 (BHQ1) and the fluorescent label is 6-carboxy-fluorescein (FAM).

Hybridization of caged and native ODNs with molecular beacon was performed as follows: 630 ng of native (noncaged), caged or caged-flashed ODN were mixed with 300 ng of complementary molecular beacon (3.6:1 ODN:Beacon molar ratio) in 100 μL solutions of 100 m*M* NaCl and 1 m*M* EDTA pH 7.5. The mixtures were denatured at 90°C for 5 min and allowed to slowly cool to 25°C over 60 min.

Fluorescence measurements of molecular beacons. Fluorescence of hybridization solutions was used to quantify the hybridization of molecular beacons with ODNs. Each of the 100 μL hybridization mixtures was excited at 492 nm and emitted fluorescence quantified at 515 nm in triplicate with a LS55B Luminescence Spectrophotometer (Perkin Elmer, Wellesley, MA). Caged ODN alone in solution was also analyzed for fluorescence at these wavelengths. To determine any possible effects of attached and photoactivated DMNPE on the fluorescence emissions of the molecular beacon, native adenosine triphosphate (ATP; 0.021 μ*M*, Sigma), DMNPE-caged ATP (0.021 μ*M*, Molecular Probes) or photoactivated DMNPE-caged ATP was added to samples with and without ODN and molecular beacon and scanned as described above.

A functional quantum yield (Φ) was calculated, based on the increases in fluorescence from the molecular beacon to signal conversion of caged ODNs, based on the following equation:

$$\Phi = \frac{1}{I\ \varepsilon\ t_{90\%}} \qquad (1)$$

where I is the irradiation intensity in moles of photons cm^{-2} s^{-1}, ε is the decadic extinction coefficient of DMNPE in cm^2 per mole of substrate and

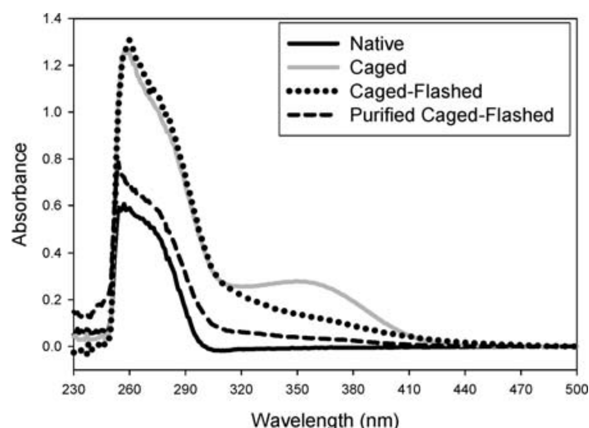

Figure 2. Spectral scans of DMNPE-caged, caged-flashed, purified caged-flashed and native oligonucleotide (ODN). All four samples were dissolved in 33% DMF and scanned for absorbance from 230 to 500 nm.

$t_{90\%}$ is the irradiation time in seconds for 90% conversion (30). A value of 4795 $M^{-1}cm^{-1}$ was used for the 365 nm molar extinction coefficient for DMNPE that was calculated as described in *Spectral scanning protocol and DMNPE extinction coefficient determination*.

Nondenaturing gel electrophoresis of hybridization products. Standard nondenaturing gel electrophoresis was used to confirm hybridization of caged and caged-flashed ODNs with complementary ODNs. A 34mer native ODN with sequence identical to the molecular beacon except that the 3′ and 5′ stem ends were replaced with thymidines (tttttttTGACGGATGC-CAGCTTGGGCttttttt) was hybridized with caged or caged-flashed ODN in the same ratios and in the same manner as described in the molecular beacon fluorescence assay. An aliquot of the mixtures (500 ng) was then run at 70 V in 15% nondenaturing polyacrylamide in TE buffer (4 m*M* tris-borate, 0.1 m*M* EDTA pH 8.5) for 100 min. Gels were stained as described in the denaturing gel electrophoresis assay. Line profile intensities of gel images were created by averaging values greater than five pixels high by the full length of each lane of the gel using ImageJ software (NIH, Bethesda, Maryland).

RESULTS

Absorbance spectrophotometry

Figure 2 shows the absorbance spectra of DMNPE-caged ODN, caged-flashed (caged-light exposed) ODN, caged-flashed-filtered and native (noncaged) ODN. Low absorbance values below 250 nm in all samples can be attributed to DMF, which has a large extinction coefficient at these wavelengths. Unlike native ODN, DMNPE-caged ODN has a characteristic absorbance peak at 355 nm, consistent with the attachment of the DMNPE cage compound (11). Based on the extinction coefficient of attached DMNPE, an average number of DMNPE caging groups per ODN was calculated as described previously, but here we also account for the 260 nm absorbance of DMNPE due to the greater degree of DMNPE attachment to ODNs required to inactivate hybridization (3). Absorbance at 355 nm indicates that caged ODN has approximately 14–16 DMNPE cage groups per 20mer ODN. Once flashed, a shift can be noted in the 355 nm peak that broadens toward longer wavelengths. The absorbance of the released cage group prevents estimation of photoactivation by spectrophotometry, so some samples were purified to remove the released cage and then scanned again. The purified caged-flashed ODN product has an even lower absorbance with a similar trend in the range of 350 to 370 nm. The caged-flashed-filtered ODN has approximately 2–4 DMNPE cage groups per ODN.

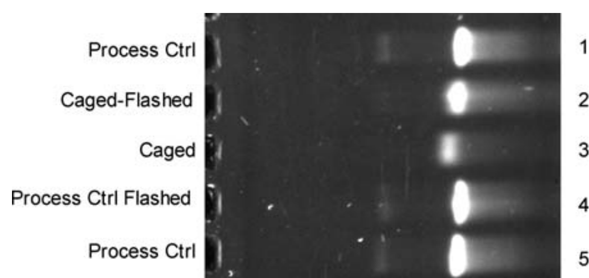

Figure 3. Denaturing gel electrophoresis of DMNPE-caged and native ODNs. Caged-flashed samples were exposed to 5.6 J/cm^2 of 365 nm light before electrophoresis. A process control lane contains ODN that was exposed to the caging reaction conditions but did not contain the cage compound.

Denaturing gel electrophoresis

Electrophoresis of caged ODNs shows characteristic changes in mobility corresponding to the addition and removal of the DMNPE caging groups (Fig. 3). The DMNPE-caged ODNs (lane 3) have reduced mobility compared to that of the control ODN (lanes 1 and 5). For comparison, a process control ODN was subjected to caging conditions and processes of the caging reaction, but without the addition of DMNPE. A light-induced change is observed between the caged and caged-flashed samples, with the caged-flashed band (lane 2) having mobility comparable to the process control ODN. The intensity of the band corresponding to the caged ODNs was also less than the caged-light-exposed and native ODNs, suggesting an alteration of the ODN that interferes with its staining. Neither mobility shifts nor staining differences are apparent when the process control sample is exposed to light.

Fluorescence measurements of hybridization products

Fluorescence emissions from a complementary molecular beacon indicate differing amounts of hybridization for caged and native ODNs (Fig. 4). Relative fluorescence units of molecular beacon in solution alone was 34.6 ± 9.4 (mean ± SD, n = 3) and 414 ± 34.3

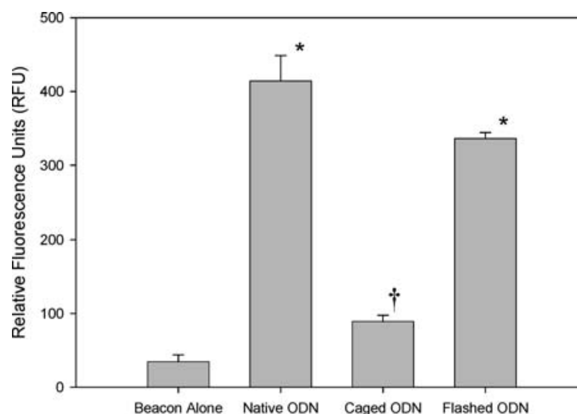

Figure 4. Relative fluorescence intensities from solutions of molecular beacon hybridized with native, caged or caged-light-exposed ODNs. Native, caged or caged-light-exposed ODN (630 ng) was mixed with molecular beacon (300 ng) and tested for hybridization in a fluorescence spectrofluorimeter (samples correspond to depictions A–D in Fig. 1). Significant difference from beacon alone is denoted by the cross symbol, whereas asterisks denote significant difference from both caged ODN and beacon alone (n = 3, $P < 0.05$).

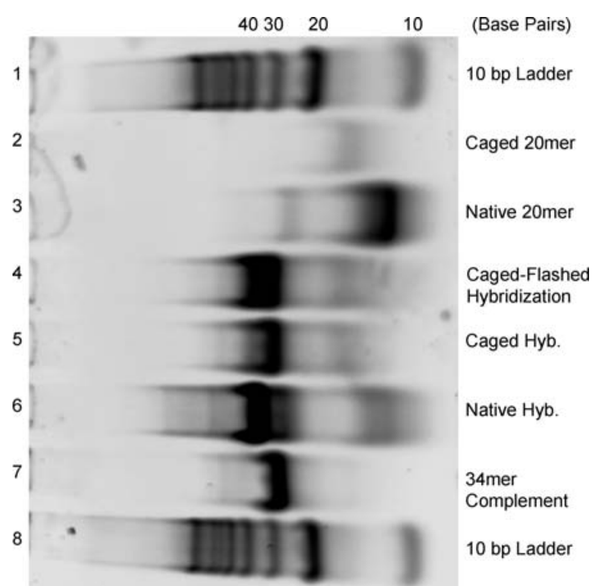

Figure 5. Nondenaturing gel (inverse image) of caged and caged-flashed ODNs hybridized with a complementary ODN. Electrophoretic patterns of native, caged and caged-flashed 20mer ODNs with and without and 34mer complementary ODNs were analyzed for changes in intensity of the duplex and ssDNA bands.

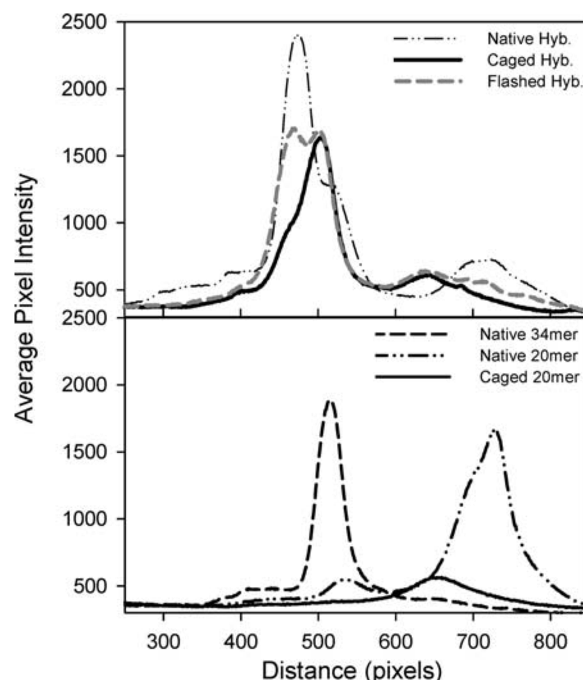

Figure 6. Line profiles of pixel intensity from the image of the gel in Figure 5. Top panel shows differences in intensity and location of bands from hybridized mixtures of native, caged and caged-flashed 20mer and 34mer ODNs. Bottom panel shows patterns of the native and caged 20mer and native 34mer ODNs.

when native compliment was added, corresponding to depictions (A) and (B) in Figure 1. Fluorescent emission of the hybridization mixture of caged ODN and molecular beacon is 89.2 ± 8.3, which is 14.4% of the relative fluorescence of the native probe hybridization mixture when background signal of molecular beacon alone in solution is subtracted, indicating a low level of hybridization ([C] in Fig. 1). However, the caged-light-exposed sample shows an increase in fluorescence to 336 ± 8.0, which is 79.5% of the native solution, indicating an increase in hybridization of the photoactivated ODNs ([D] in Fig. 1). Significant difference from beacon alone is denoted by the cross symbol, whereas asterisks denote significant difference from both caged ODN and beacon alone (n = 3, $P < 0.05$, *t*-test between means). DMNPE-caged ODNs alone in solution have no fluorescent emissions at these excitation wavelengths. The presence of DMNPE-caged or DMNPE-caged-flashed ATP in solution with native or caged ODN hybridizations did not significantly alter fluorescence intensity (data not shown).

Nondenaturing gel electrophoresis of hybridization products

To confirm the results from the solution measurements of molecular beacon hybridization, nondenaturing polyacrylamide electrophoresis was used to show gel mobility shifts upon hybridization with caged and caged-light-exposed ODNs. Single-stranded (ssDNA) ODN, a 34mer ODN complementary to the caged ODN, has slower mobility than the 20mer native and caged ODNs run separately (Fig. 5, lanes 7, 2 and 3, respectively). Hybridized duplexes of the 34mer and native 20mer ODNs have slowest mobility (lane 6, darker band), similar to that of a 35 base pair fragment when compared to the ladder (lanes 1 and 8). Characteristic shifts in banding patterns can be seen between the caged and caged-flashed hybridization mixtures (lanes 5 and 4). The lane containing the caged hybridization sample has a less intense band corresponding to the duplex conformation, and a stronger band of the ssDNA 34mer ODN alone. Caged-flashed mixtures have a greater intensity in the duplex band, more resembling the native hybridization mixture. Both the caged and caged-flashed samples have bands resembling the caged 20mer that did not hybridize with the complementary 34mer ODN.

Line profile analysis of the hybridization gel image in Figure 5 can be seen in Figure 6. Image analysis on the bands in the separate lanes shows distinct changes seen in position and intensity between native, caged and caged-flashed hybridized samples. The native hybridization lane has peak intensity at 474 pixels, whereas the 34mer ODN alone has a peak at 515 pixels. The caged sample lane has a peak at 505 pixels, and the caged-flashed sample lane has a doublet with peaks at 467 and 504 pixels respectively. The caged and flashed hybridization sample lanes also have smaller peaks around 660 pixels, similar to the caged sample alone. The native hybridization sample lane also has a smaller peak corresponding to the excess native 20mer around 727 pixels, similar to the peak seen in the lane containing only native 20mer ODN. Intensity counts corresponding to the hybridized peak position at 467 pixels were 1003, 1703 and 2336 counts for the caged, caged-flashed and native hybridized samples, respectively.

Light dose-response of caged ODNs and molecular beacons

To determine the proper dose of light exposure required for photoactivation of caged ODNs, hybridization mixtures were exposed to increasing durations of light before heat-denaturing and fluorescence measurement (Fig. 7). Fluorescence emission increased with increasing light exposure, from 230 RFU with no light exposure, to values of 302, 325, 344, 372 and 380 RFU with light exposures of 1, 2, 5, 10 and 20 min, respectively. Significant

difference from unexposed caged ODN are denoted by asterisks (n = 3, $P < 0.05$, Bonferroni *t*-test).

DISCUSSION

Our data suggests that the photocaging of DNA oligonucleotides (ODNs) can reversibly block hybridization. This strategy relies on the covalent attachment of a cage compound, which disrupts DNA hybridization until photocleavage restores DNA to its native and bioactive form. We have partially blocked ODN hybridization with the cage compound DMNPE, the same compound that has been shown to reversibly control transcriptional activity of plasmid DNA (3).

Absorbance data and gel shifts indicate that DMNPE forms adducts with ODNs and is photocleaved with 365 nm light. Spectral scans show characteristic absorbance at 355 nm (Fig. 2). Similar to DMNPE-caged plasmid DNA and DMNPE-caged ATP, absorption at this wavelength is consistent with the presence of DMNPE caging groups, as native DNA does not absorb in this region. Calculations based on the extinction coefficient of attached DMNPE indicate that there are 14 to 16 cage molecules present per 20mer ODN. The spectral scans in Figure 2 also indicate that photocleavage of the caged ODNs was achieved. The purification of the flashed products (purified flashed-filtered) confirms this as the absorbance at the 355 to 390 nm range decreases once the sample was filtered. By filtering the caged-flashed product, released cage could be removed, and thus a more effective characterization of the flashed product was possible. In gel electrophoresis, caged ODNs have lower electrophoretic mobility than native (noncaged) ODNs (Fig. 3). This observation is consistent with the attachment of the nonpolar DMNPE cage groups that retard ODN mobility by neutralizing otherwise negative charges on the phosphodiester backbone or base structures. It was originally hypothesized that the DMNPE attaches to the phosphate backbone of DNA, similar to its known attachment to phosphates of nucleotides (11). While structural studies of the DMNPE-caged ODNs have not been completed to date, alterations in staining intensity between caged and native ODNs (lanes 2 and 3) suggest that attachment of the DMNPE may also block some reported base-associated labeling of the SYBR-Gold nucleic acid stain used to visualize these ODNs in gels (31). Even if the majority of DMNPE attachment occurs at the phosphate backbone of the ODN, this conformation may still disrupt hybridization, as shown with other phosphate modifications (32).

Two assays demonstrate that caging ODNs modifies their hybridization activity. The switch-like fluorescence properties of the molecular beacon allow it to act as a direct measure of hybridization, so that solutions of hybridized ODN and beacon can be assayed for hybridization directly in a spectrofluorimeter. Fluorescence emission of a hybridization mixture of caged ODNs and complementary beacon is much lower than that of the native hybridization mixture (Fig. 4). When exposed to 5.6 J/cm^2 of 365 nm light before hybridization, fluorescence emission increases, indicating a restoration of hybridization activity. When the background of native probe alone is subtracted, the level of activity between the caged and caged-light-exposed ODN-beacon hybridization increases from 15% to 79% of the native ODN hybridization activity. Quenching or other effects of DMNPE on molecular beacon fluorescence were ruled out, as were fluorescence emissions from DMNPE-caged ODN itself. To corroborate the results found in solution, a nondenaturing polyacrylamide gel

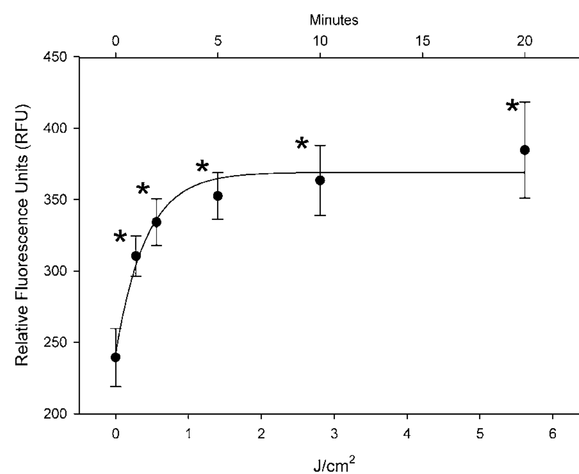

Figure 7. Effect of cumulative light exposure time on solution fluorescence of caged ODN-molecular beacon hybridization mixtures. Significant difference from unexposed caged ODN are denoted by asterisks (n = 3, $P < 0.05$).

electrophoresis shows shifts in gel bands upon hybridization (Fig. 5). Decreased intensity of the band representing the duplex hybridization of 20mer ODN and 34mer complementary ODN shows that caged ODN does not completely hybridize. However, when exposed to 365 nm light before hybridization and electrophoresis, cage groups are photocleaved from the ODN allowing it to hybridize. The caged-light-exposed sample has a stronger band in the duplex conformation resembling that of the noncaged native probe hybridization. The presence of a stronger band of the 20mer nonhybridized ODN in the caged sample that is not present in the flashed sample also confirms the alteration of hybridization activity observed in solution. More quantitative image analysis of these gels was performed by generating line profiles that show electropherogram-like profiles seen in Figure 6. These line profiles indicate the large differences in hybridization between native, caged and caged-flashed samples. The caged hybridization sample has only a peak similar to the complementary target alone, but when exposed to light, shows a doublet peak that corresponds to the duplex banding pattern seen in the native sample, indicating changes in hybridization activity with light exposure.

Restoration of hybridization of caged ODNs is a light-dependent process as seen in the 365 nm light dose response of hybridization activity in Figure 7. A hyperbolic relationship is seen between the amount of fluorescence from a molecular beacon and increasing light exposure, with a dose one-half of 0.288 J/cm^2. It is difficult to compare this dose of light with other photolysis studies because most caged compounds are directly synthesized with only one cage moiety per effector molecule. However, the doses for functional restoration of DMNPE-caged ODNs do appear to be within the range of other published values of photolysis for similar cage compounds and light delivery systems (33,34). The caging group used here, DMNPE, in general has relatively low quantum yields compared to other caged groups. For instance, the reported Qp for DMNPE-caged ATP is 0.07, much lower than the 0.63 Qp of NPE-caged ATP (11,35). An attempt to estimate the quantum yield of DMNPE-caged ODNs was made based on the increase in functional activity with light exposure in the molecular beacon assay in Figure 4. The measured increase in hybridization was used to determine complete conversion rather than the traditional method

of quantum yield determination from absorption increases. The fact that each caged ODN has multiple DMNPE adducts precludes the simple use of an absorption increase because restoration of hybridization may not correlate with the photoconversion of less than all the attached cages. Our calculations indicate that DMNPE-caged ODNs have a functional quantum yield of at least 0.09. This number assumes a 100% response from the molecular beacon to indicate hybridization of a photoreleased ODN, which may not be the case. Lower efficiencies of the molecular beacon system in detecting hybridization of a photoactivated ODN would impart increases to the resulting quantum yield.

Modifications of the strategy could lead to improvements in blockade and subsequent restoration of hybridization of ODNs. Since our initial report of caging plasmid DNA with DMNPE, other promising cage groups have been identified. For example, brominated 7-hydroxycoumarin-4-ylmethyls (BHC), a modification of the Tsien bromocoumarin cage, has recently been used to randomly cage mRNA, allowing it to be activated at selected sites for translation in zebrafish embryos, facilitating the elucidation of several genes involved in development (24). BHC reportedly has better quantum efficiencies than DMNPE, which would require less light for complete photocleavage after delivery to cells (36). In addition to a lower light dose, the architecture of light exposure to tissue could be engineered to minimize cellular-induced responses, while maximizing total dose of light delivered to the caged ODNs. Two-photon excitation could be an alternative technique for photoactivation of caged ODNs (37). Longer wavelengths utilized in the two-photon uncaging have lower energy than the 365 nm light, causing less photodamage to cells and tissues (38). The longer wavelengths also have deeper tissue penetration and would allow targeting at greater depths. This technique, combined with newer cage molecules having biologically useful 2-photon cross-sections, could minimize cellular damage while increasing targeting precision (39).

In summary, our data indicate that light-activated hybridization can be achieved with the use of photocleavable cage compounds. Spectrophotometric, gel-shift and molecular beacon fluorescence data indicate that caged compounds can be used to reversibly alter the hybridization activity of single-stranded DNA. Attachment of DMNPE to phosphodiester ODNs presents a strategy for the temporal and spatial control of hybridization. This strategy has application in controlling DNA hybridization activity such as primer activity in PCR, fluorescence in situ hybridization, micro-arrays, molecular computers, nanomachines, DNA-biosensors and targeting of antisense ODNs to specific locations in tissues. Extension of this technique to phosphorothioate ODNs and RNAi species could be applicable in controlling gene silencing and protection of these compounds from enzymatic degradation.

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