Photoactivatable Compounds

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Nucleic Acid Binders Activated by Light of Selectable Wavelength**

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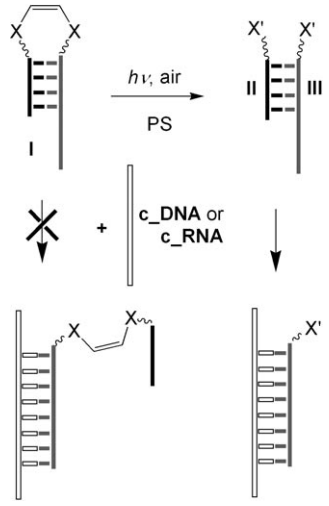
In the cell oligonucleotides and their analogues bind natural RNA in such a way that, in some cases, RNAse H-catalyzed RNA cleavage ensues. This process (antisense effect) affects gene expression, for example, by inhibiting mRNA maturation or translation. An alternative indirect mechanism includes binding and blocking microRNAs, which are natural regulators of gene expression.[1] Antisense oligonucleotides are utilized in biological studies for the determination of gene functions and in medicine for the suppression of disease-related genes.

"Caged" or photoactivatable oligonucleotides are chemically modified compounds that are poor RNA binders until they are "uncaged" or photoactivated by light.[2] They may be used for spatially and temporally controlled photoregulation of gene expression. Many approaches for the preparation of caged antisense DNAs have been reported.[2] For example, Komiyama and co-workers introduced several unnatural fragments containing *trans*-azobenzene units in the middle of a DNA sequence.[3] These conjugates tightly bind and, therefore, deactivate antisense DNAs. Upon irradiation with UV light the *trans*-azobenzene units are isomerized into duplex-destabilizing *cis* derivatives. This leads to release and reactivation of the antisense DNAs. Tang and Dmochowski replaced one nucleotide in the loop of a DNA hairpin with a photocleavable carbamate linker. This caged DNA does not promote RNAse H-catalyzed hydrolysis of complementary RNAs unless the linker is cleaved by UV light.[4] Other backbone modifications that can be cleaved by UV light have been reviewed recently.[2] DNA caging is also possible by modifications of phosphodiester groups[5] and nucleobases.[6]

Photoactivation of caged antisense DNAs in cells has not been demonstrated yet. In contrast, the biological effects of caged mRNA, plasmids, and small interfering RNAs (siRNA) have been already shown. For example, mRNA-coding Gfp conjugated with several 6-bromo-7-hydroxycoumarin units is weakly translated in zebrafish embryos in the dark. Upon exposure to the light (350–365 nm) the chemical modifications are cleaved and the natural mRNA is formed. Translation of the resulting mRNA becomes more pronounced, as evidenced by elevated levels of Gfp.[7] In another study a plasmid coding for Gfp was modified with 1-(4,5-dimethoxy-2-nitrophenyl)diazomethane. The caged plasmid shows 25.8% of native plasmid expression in HeLa cells in dark. After irradiation with 355-nm light, the Gfp expression level is increased to 50% with respect to the control.[8] Friedman and co-workers used a similar strategy for caging siRNAs.[9] In practically all reported examples uncaging is done with UV light.[2] Light of this type is strongly absorbed by cellular components and is damaging to the cells.[10] UV light itself may affect gene expression. For example, Haselton and co-workers observed that UV light ($>0.5\,J\,cm^{-2}$) inhibits expression of pEGFP-C1 plasmids in HeLa cells.[8] This limits applications of caged antisense agents.

Herein we describe DNAs that can be activated by light in any chosen spectral region, including, for example, red light. Red light is significantly less harmful than UV light and can permeate deeply into tissues.[11] Our concept of caged oligonucleotides is presented in Scheme 1.



Scheme 1. The concept of a caged antisense agent (**I**), which can be activated by light in any chosen spectral region; PS: photosensitizer, **c_DNA**: DNA complementary to the gray sequence of **I**, **c_RNA**: RNA complementary to the gray sequence of **I**, **X**: electron-donor group or atom (e.g. S); *hν*: light absorbed by the PS.

Caged DNA **I** is not a binder of single-stranded (ss) nucleic acids complementary to its recognition sequence (gray), since the latter is blocked by a short DNA strand (black). The loop of **I** contains an electron-rich C=C bond. In the presence of a photosensitizer (PS) and upon illumination with light, singlet oxygen ($^1O_2$) is produced[12] which induces cleavage of the C=C bond and formation of an intermolecular DNA duplex **II**·**III**. Duplex **II**·**III** is substantially less stable

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Supporting information for this article, including descriptions of the preparation and characterization of compound **3** and DNAs **I** and **I-E**, is available on the WWW under http://www.angewandte.org or from the author.

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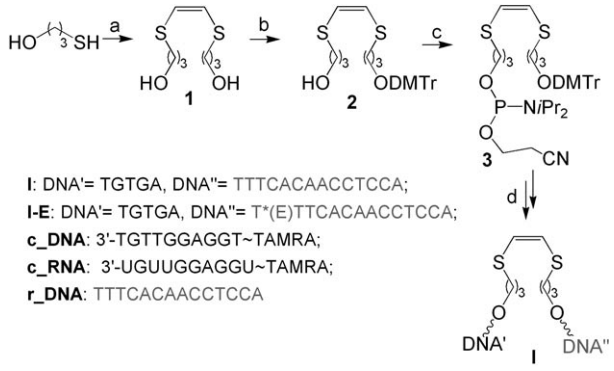
than the initial hairpin structure. A complementary DNA (or RNA) can replace strand **II** to form a full-length duplex **III·c_DNA** (or **III·c_RNA**). Thus, light triggers the hybridization process.

We chose SCH=CHS as a singlet-oxygen-sensitive group. Breslow and co-workers reported earlier that the C=C bond in this fragment is quickly and cleanly cleaved by singlet oxygen in aqueous solutions.[13] DNA containing SCH=CHS (**I**, Scheme 2) was prepared by standard solid-phase DNA



Scheme 2. Synthesis of caged DNA **I**: a) *cis*-1,2-dichloroethene, NaOH, b) DMTr-Cl, NEt_3, DMAP, c) $NC(CH_2)_2OP(Cl)N{i}Pr_2$, tetrazole, d) automated DNA synthesis. T* is a modified T: 5-[aminohexyl)-3-acrylimido]-2′-deoxyuridine; in T*(E) the amino groups are conjugated to eosin. DMAP = 4-(dimethylamino)pyridine; DMTr-Cl = 4,4′-dimethoxytrityl chloride; TAMRA = 5-/6-Carboxytetramethylrhodamine.

synthesis; all starting materials are commercially available, except for phosphoramidite **3**, which we prepared by a three-step procedure (Scheme 2). It couples with > 95 % efficiency under standard conditions for DNA synthesis.

We first tested eosin as a PS ($\lambda_{max} \approx 525$ nm in air-saturated aqueous solution of 3-morpholinopropane sulfonic acid (MOPS), pH 7). The triplet excited state of eosin is populated upon illumination with green light. The excited dye relaxes back to its ground state by energy transfer to triplet oxygen, forming singlet oxygen.[14] We detected formation of $^1O_2$ by monitoring decomposition of an oxygen trap, 1,3-diphenylisobenzofuran (decrease of absorption at 415 nm).[15] In the presence of DNA **I** photogenerated singlet oxygen reacts with the C=C bond in the DNA loop forming the product of [2 + 2] addition. This compound then decomposes into in two fragments containing terminal thioester groups: DNA′ ~ SC(=O)H and DNA″ ~ SC(=O)H. In aqueous solution the thioesters are partially hydrolyzed to form DNA′ ~ SH (**II**) and DNA″ ~ SH (**III**), respectively. The DNAs containing SH groups are oxidized with formation of an unsymmetrical disulfide (DNA′ ~ S-S ~ DNA″). Symmetric disulfides are not obtained. This indicates that preorganization of the SH groups in the **II·III** duplex, formed as a result of DNA **I** cleavage, is required for the disulfide formation. DNA′ ~ S-S ~ DNA″ is obtained even when the reaction is conducted in the presence of twofold excess of **c_DNA**. Apparently the oxidation is a much faster process than duplex dissociation. The disulfide DNA as well as the DNAs containing SC(=O)H can be transformed into HS ~ DNAs by treatment with 10 mM dithiothritol (DTT; Figure 1). The minor by-product having mass [*M* + 16] (where *M* is molecular mass of DNA **I**) is also formed as the result of the

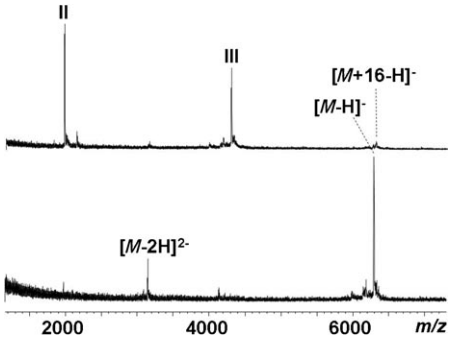


Figure 1. MALDI-TOF mass spectra of DNA **I** (5 μM) in NH_4OAc buffer (100 mM, pH 7) containing eosin (1 equiv). Bottom spectrum was acquired from the solution kept for 90 min in dark; upper spectrum was acquired from the solution irradiated for 90 min with green light (15-W halogen lamp and green-light filter). After the irradiation both solutions were treated with DTT (10 mM) for 24 h to convert the thioesters and disulfides into thiols. [*M*]: molecular ion peak of DNA **I**; DNAs **II** and **III** are fragments resulting from cleavage of **I** (see Scheme 1).

cleavage. This product is not sensitive to DTT treatment and is formed only from DNAs containing SCH=CHS fragment. We speculate that it is the sulfoxide resulting from the addition of oxygen to one of the sulfur atoms of SCH=CHS. The oxygen may come from peroxo species formed in the reaction of singlet oxygen with reductive agents, for example, water.

The C=C bond in DNA **I** is also cleaved when chlorine-e6 ($\lambda_{max} \approx 410$ and 650 nm) is used instead of eosin and red light instead of green one. This indicates that C=C bond cleavage does not depend on the type of PS and light but rather correlates with formation of $^1O_2$. Variation of PS may allow quick tuning of the system for specific applications. In contrast, tuning possibilities for reported caged oligonucleotides are rather limited.[2]

Thiols are present in high concentrations in cells. They are important antioxidants and can, for example, scavenge singlet oxygen. To model the environment within the cell we added large amounts of DTT (10 mM) to the reaction buffer. Cleavage of DNA **I** was not fully inhibited at these conditions (Table 1). Moreover, formation of the disulfide and thioesters

Table 1: HPLC analysis of photocleavage of caged DNAs.

Run	Reaction mixture[a]	DNA cleavage [%][b]	
		in the dark	with irradiation
1	DNA **I**, eosin	8	92
2	DNA **I**, eosin, DTT	11	46
3	DNA **I**, chlorine-e6	9	67
4	DNA **I**, chlorine-e6, DTT	2	28
5	DNA **I-E**, DTT	7	91

[a] [DNA] = 5 μM, [PS] = 5 μM, 100 mM NH_4OAc buffer, pH 7; runs 1–4 were irradiated for 90 min, and run 5 was irradiated for 150 min; after the irradiation runs 1 and 3 were treated with DTT (10 mM) for 24 h. [b] 100 % [**II**]/$[DNA]_{t=0}$.

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was suppressed and, consequently, the yield of SH-containing fragments **II** and **III** increased. This indicates that **I** can be potentially photoactivated in cells. Unfortunately, a competing reaction, the formation of the $[M+16]$ by-product was substantially accelerated in the DTT-containing buffer. This is in agreement with our identification of this compound. In particular, at high DTT concentrations and, therefore, under more reducing conditions, the quenching of $^1O_2$ and formation of peroxo species are facilitated. Correspondingly, the yield of sulfoxide should increase. Although DNA (deoxyribose, nucleobases) can react with singlet oxygen and its decomposition products, we did not detect any products of unspecific DNA modification or cleavage at our experimental conditions.

The sequence of DNA **I** was designed in such a way that in solution it spontaneously folds into the hairpin conformation (Scheme 1). This hairpin is a poor binder of complementary single-stranded nucleic acids. In particular, only about 40% of DNA **I** is hybridized with **c_DNA** in buffered aqueous solution at pH 7 (lanes 2 and 4 in Figure 2). Under similar

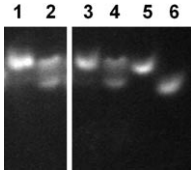

Figure 2. Gel electrophoresis under native conditions. In all lanes: **c_DNA** (4 μM), acetate buffer (10 mM), pH 7, DTT (7 mM), NaCl (1 M). Lane 1: DNA **I** (5 μM), eosin (1 equiv), green light for 90 min; lane 2: same as for lane 1 but without irradiation; lane 3: DNA **I** (5 μM), chlorine-e6 (1 equiv), red light for 90 min; lane 4: same as for lane 3 but without irradiation; lane 5: **r_DNA** (positive control); lane 6: no DNA or RNA present besides **c_DNA** (negative control). Before analysis the solutions were treated with DTT (10 mM) for 24 h.

conditions, 100% of a control ss DNA (**r_DNA**) binds to **c_DNA** (lane 5). Irradiation of DNA **I** followed by DTT treatment converts 67% (chlorine-e6) and 92% (eosin) of DNA **I** to DNAs **II** and **III**. The latter forms stable duplex with **c_DNA**, as is indicated by gel electrophoresis data (Figure 2).

The biological targets of antisense agents are natural RNAs. Therefore, we conducted the same experiment with a complementary RNA (**c_RNA**). As in the case of the DNA target, the binding of DNA **I** to **c_RNA** could be photoactivated by both green (PS = eosin) and red light (PS = chlorine-e6). When the photoactivation of DNA **I** was performed in a DTT-containing buffer, the yield of DNA **III** and **II** was reduced by approximately a factor of 2 (Table 1). The main side product is the sulfoxide of DNA **I** ($[M+16]$ product). Like DNA **I**, the sulfoxide is a poor binder of ss nucleic acids. To increase the efficiency of the uncaging of DNA **I** under physiological conditions we prepared the analogue DNA **I-E**, which contains a PS covalently attached to the DNA in proximity to SCH=CHS group. This compound was synthesized similarly to DNA **I**, except that the amino-modifier C6 dT (Glen Research) was introduced to DNA″ in place of T1, and the resulting amino-modified DNA was conjugated with eosin isothiocyanate[14] (Scheme 2). DNA **I-E** is efficiently activated by green light even in the DTT-containing buffer. In particular, the yield of DNA **I-E** photocleavage products is 91%. The substantial improvement with respect to DNA **I** can be explained by the proximity of the $^1O_2$-generating center (eosin) to the SCH=CHS group. This facilitates the direct reaction of singlet oxygen with the double bond, while $^1O_2$ quenching with DTT is less affected.

Mitochondria in cells produce a series of reactive oxygen species (ROS), including also singlet oxygen. ROS concentration in normal cells is kept low by antioxidants. However, near the mitochondria the local concentration of $^1O_2$ may be high. This could lead to the spontaneous activation of our caged DNAs in the absence of light. Ongoing tests of the caged DNAs in cells will show whether this is a serious problem.

We have prepared DNA derivatives whose nucleic acid binding properties can be efficiently triggered by either green or red light. This is a general approach. In particular, light in any other spectral region can be potentially utilized for activation of these nucleic acid binders providing a suitable photosensitizer is used. Conjugation of photosensitizers with caged DNAs improves their properties substantially.

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