Light-triggered polymerase chain reaction

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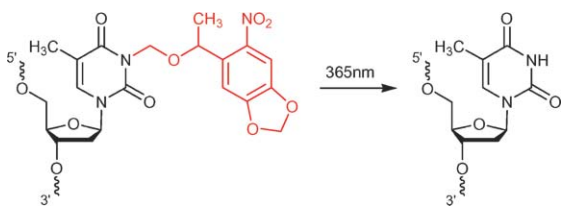
Photochemical control of the polymerase chain reaction has been achieved through the incorporation of light-triggered nucleotides into DNA.

Photochemical activation enables the precise spatial and temporal regulation of chemical and biological function. This is typically achieved through the installation of photochemically removable protecting groups (caging groups) on the molecule of interest, often a biological macromolecule. These caging groups are then removed in a spatially and temporally restricted fashion through irradiation with UV light (decaging), leading to activation of the molecule under study.[1] Caging has been employed in the photochemical regulation of several processes, *e.g.* enzymatic activity,[2] gene expression,[3] as well as DNA and RNA function.[4,5] Here, we report on the photochemical regulation of the polymerase chain reaction (PCR). PCR was developed in 1983, and is employed in the *in vitro* isolation and exponential amplification of specific DNA sequences.[6] By utilizing thermophilic DNA polymerases with specifically designed DNA primers, extremely small amounts of DNA can be rapidly enriched to substantial quantities. In the few years since its discovery, PCR has revolutionized the field of molecular biology, facilitating genome sequencing, genetic disease diagnosis, and genetic fingerprinting.[7] We expect that the photoregulation of PCR will afford an additional level of control over this important technique.

Recently, we developed a novel caging group for *N*-heterocyclic molecules[8] and applied it to the specific caging of a thymidine nucleotide on its heterocyclic base.[4] The corresponding caged phosphoramidite was incorporated into DNA oligomers using standard DNA synthesis equipment and protocols.[4] Installation of the sterically demanding caging group in conjunction with the disruption of an N–H bond critical for Watson–Crick base pairing allowed for the attenuation of catalytic activity of a DNAzyme. Brief irradiation with UV light of 365 nm (25 W, handheld UV lamp) removes the caging group and generates the regular DNA oligomer (Scheme 1). In order to apply this approach to the photochemical regulation of PCR, we first investigated the effect of one or multiple caging groups on the hybridization to a complementary DNA strand. The DNA oligomers **P1–P7**, consisting of 19 nucleotides, a typical length for PCR primers, and containing 0–4 caged thymidines have been synthesized (Table 1). These primers were then analyzed for their annealing and melting properties in the presence of a complementary oligonucleotide.

Melting curves were measured on a BioRad MyiQ RT-PCR thermocycler by conducting a sequence of 3 heating and cooling cycles (1 μM of both primer and complementary DNA with 12.5 μL iQ SYBR Green Supermix to a total volume of 25 μL; 40 °C to 80 °C with a 0.5 °C min^{-1} ramp). The melting temperatures were determined to be 65.3 °C (**P1**), 62.1 °C (**P2**), 54.3 °C (**P3**), 55.5 °C (**P4**), and 50.0 °C (**P6**). No melting temperatures could be measured for **P5** and **P7**, leading to the assumption that no hybridization occurs. These results indicate that both the number of caging groups and the position of the caged thymidine residues affect DNA hybridization. Installation of a single caging group results in a melting temperature depression of 3.2 °C and 11.0 °C as seen in **P2** and **P3**, respectively. This effect is less pronounced in **P2** perhaps due to the caged T's close proximity to the 5′ terminus, leading to a lower level of interference with the hybridization of neighboring nucleotides. Very similar melting point depressions and positional variations have previously been observed in T-mismatches.[9] With the incorporation of additional caging groups in **P4–P7**, melting temperatures decrease further. However, addition of a single caged thymidine close to the 5′ terminus of **P3** had no effect in **P4**. A positional effect was also observed with three caging groups, as seen in **P5** and **P6**. The primer **P6** contains a cluster of three caged thymidines and displays a higher melting temperature than **P5** containing three caging groups distributed throughout the DNA oligomer, thus leading to a more effective disruption of hybridization. In order to ensure a complete removal of the photolabile group, each primer was irradiated for 8 min at 365 nm (Scheme 1), and then analyzed in the same melting temperature assay. As expected, irradiation led to full restoration of DNA hybridization, as each primer displayed a comparable melting temperature to the non-caged analog **P1**.

These experiments revealed that the presence of three caging groups distributed evenly throughout a 19 nucleotide oligomer is sufficient to disrupt hybridization and thus will prevent annealing of a PCR primer to its cognate DNA template, at the typical annealing temperature range of 50–65 °C.[7] The stability of the caging group to PCR conditions was examined on the monomeric caged thymidine, and found to be unaffected by the elevated temperatures required for PCR (data not shown). These results set the stage for the application of **P5** in a light-activated PCR experiment (Scheme 2).



Scheme 1 Decaging of DNA containing the caged thymidine. The light-removable caging group is shown in red.

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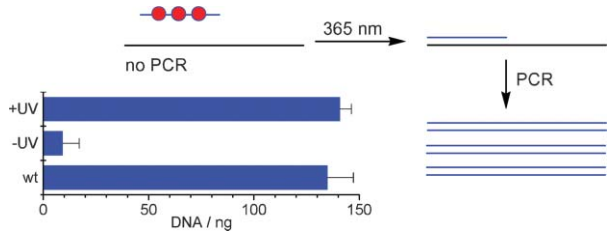
Table 1 Melting temperatures of non-caged (**P1**) and caged oligonucleotides (**P2–P7**) before and after UV irradiation (365 nm)

	DNA sequence[a]	Mp/°C −UV[b]	Mp/°C +UV[b]
P1	5′ CTGATTTCGACCAGGTTCG 3′	65.3 ± 0.3	65.0 ± 0.8
P2	5′ **C**TGATTTCGACCAGGTTCG 3′	62.1 ± 0.7	64.1 ± 0.2
P3	5′ CTGATT**C**GACCAGGTTCG 3′	54.3 ± 1.1	64.1 ± 0.8
P4	5′ C**T**GATTTCGACCAGGTTCG 3′	55.5 ± 0.5	63.8 ± 0.3
P5	5′ CTGATTTCGACCAGGT**T**CG 3′	ND	64.2 ± 0.2
P6	5′ CTGA**TTT**CGACCAGGTTCG 3′	50.0 ± 1.0	64.5 ± 0.5
P7	5′ CTGATTTCGACCAGGT**T**CG 3′	ND	64.2 ± 0.3

[a] **T** denotes the caged thymidine. [b] Melting temperatures (mp) determined with the non-caged complement (5′ CGAACCTGGTCGAAATCAG 3′). ND = not detectable.



Scheme 2 PCR activation by light. Black line: PCR template, blue line: primer, red circles: caged thymidines.

The DNA template (1.5 ng μL^{-1} of plasmid DNA) was incubated in the presence of **P5** and a reverse primer (5′ AGAGAGCTCGAGATCGCCATCTTCCAGCAGGCGCACCATTGCCCCTGT 3′, 1 μM each; the same reverse primer was used in all PCRs) with nucleotide triphosphates (dNTPs, 0.3 mM each), in Taq reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3) and water. Taq DNA polymerase (3 units μL^{-1}) was added to initiate the reaction. Prior to placement in a PCR thermocycler (Eppendorf Mastercycler) the reaction was either irradiated for 8 min at 365 nm, or maintained in the dark. An initial denaturation at 95 °C was performed, followed by 40 cycles consisting of 95 °C (30 s), 50 °C (30 s), and 72 °C (1 min), with a final extension at 72 °C (2 min). The reactions were conducted in triplicate, visualized on a 1% agarose gel, and quantified (all DNA was quantified by band integration in ethidium bromide stained agarose gels using Image Quant 5.2). A regular PCR reaction with non-caged **P1** produced approximately 140 ng of PCR product (Fig. 1, lane 5); however when caged **P5** was employed in the absence of UV irradiation, only trace amounts of product were detected (Fig. 1, lane 6). After irradiation, the function of **P5** was restored (Fig. 1, lane 7), leading to comparable amounts of PCR product as found in the

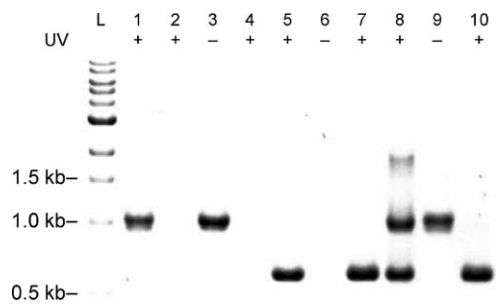


Fig. 1 Agarose gel of dsDNA amplified by light-regulated PCR.

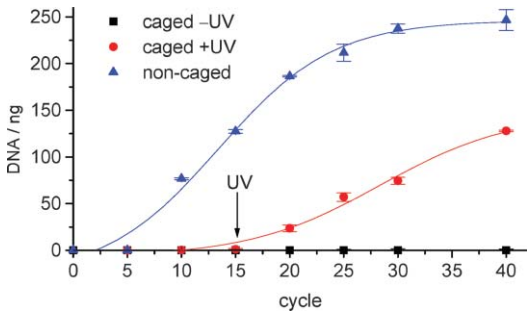
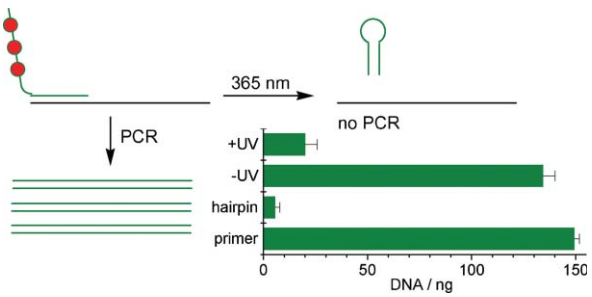


Fig. 2 Time course of a PCR light-activated at cycle 15. All experiments were done in triplicate.

reaction with **P1** (Fig. 1, lane 5). This experiment represents the first example of a light-activated PCR. While we initially employed irradiation prior to the thermal cycling, temporal control can be achieved by initiating the reaction through irradiation at a specific time point. Here, an identical reaction with UV irradiation at cycle 15 was conducted, as shown in Fig. 2. Prior to irradiation, no PCR product can be detected in reactions performed with the caged primer **P5**. In contrast, the non-caged primer **P1** leads to the expected amplification. Upon UV irradiation of the reaction with **P5** at cycle 15 the amount of DNA increases exponentially, while no amplification occurs in the corresponding non-irradiated reaction.

After achieving photochemical control of the activation of PCR at a specific time point, the possibility of switching-off PCR activity *via* light irradiation was examined. This can be accomplished by designing a self-complementary primer which is predisposed to form a hairpin, rather than act as a PCR primer. By installing caging groups on the complementary portion of this sequence it is possible to block self-hybridization, thus enabling the polymerization reaction. The PCR is then stopped by removing the photoactive groups, leading to hairpin formation and primer deactivation (Scheme 3). In order to achieve photochemical deactivation, an appropriate hairpin primer **P8** was designed (5′ GGTCAGTAAATTGTTTTTCAATTTACTGACCG 3′), and a photocaged analog **P9** was synthesized (5′ GGTCAGTAAATTGTTTTTCAATTTACTGACCG 3′). PCR using a typical primer which only possesses half of the hairpin and not its complement led to DNA amplification after 25 cycles (Scheme 3 and Fig. 1, lane 1). Conversely, the non-caged hairpin primer **P8** failed to amplify the DNA leading to very little PCR product (Fig. 1, lane 2). However, in the absence of light irradiation the caged hairpin primer **P9** was successful in acting as a primer, yielding 137 ng of PCR product (Fig. 1, lane 3). This suggests that



Scheme 3 PCR deactivation by light. Black line: PCR template, green line: primer, red circles: caged thymidines.

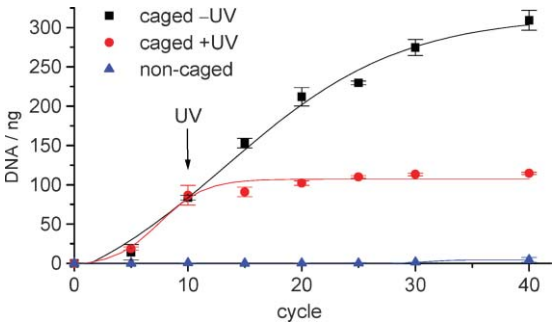


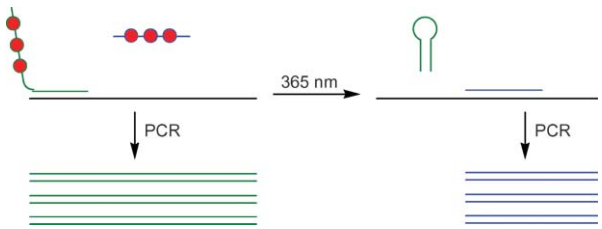
Fig. 3 Time course of a PCR light-deactivated at cycle 10. All experiments were done in triplicate.

the 3 installed caging groups prevented hairpin formation, and allowed the complementary sequence to act as a PCR primer. If **P9** was irradiated for 8 min at 365 nm, the caging groups were removed, leading to hairpin formation and suppression of DNA amplification (Fig. 1, lane 4).

These light-regulatory mechanisms could also be employed in a temporally controlled fashion by irradiating the caged primer after 10 cycles of PCR (Fig. 3). The non-caged primer **P8** was used as a control, as it formed a hairpin immediately, inhibiting amplification. Because the caged primer **P9** was able to bind to the DNA template, amplification occurred. At cycle 10 the reaction containing **P9** was irradiated leading to removal of the caging groups, hairpin formation, and effective inhibition of DNA amplification. At the same point, amplification continued in the non-irradiated reaction with **P9**.

With the two caged primers **P5** and **P9** possessing opposing effects on the PCR reaction upon light irradiation, it was possible to utilize both primers simultaneously to stop the production of one PCR product, while also triggering the amplification of a different PCR product *via* irradiation with UV light (Scheme 4). Thus, **P5**, **P9**, and the reverse primer were included in the PCR reaction mixture. A product band of ~1.0 kb was detected in the non-irradiated reaction after 20 cycles, which is attributed to the caged and inactive **P5** and the caged but active **P9** (Fig. 1, lane 9). Alternatively, a product band of ~0.6 kb was observed in the irradiated reaction, as a result of the decaging and activation of **P5** and the deactivation of **P9** (Fig. 1, lane 10). As expected, both bands were observed in the control reaction using non-caged and non-hairpin primers (Fig. 1, lane 8).

In summary, a photochemical activation and deactivation of the polymerase chain reaction has been developed. This was achieved through the incorporation of caged thymidine phosphoramidites into oligonucleotide primers using standard DNA synthesis protocols. By effectively disrupting DNA hybridization through the site-specific installation of caging groups and restoring it with light irradiation it was possible to control activation and deactivation of PCR in a temporal fashion. Moreover, by conducting a simultaneous activation and deactivation, light switching from one DNA amplification product to another was accomplished. We believe that real-time PCR thermocyclers[10] could be easily equipped with UV light sources enabling primer decaging at any time of the experiment. Non-specific amplification of DNA could be prevented by caged PCR primers. Heating the reaction mixture to the annealing temperature followed by



Scheme 4 Light-switching between two different PCR reactions through simultaneous activation and deactivation of primers.

irradiation would then constitute a simplified hot-start PCR protocol.†

Notes and references

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