Photochemical Gene Control

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Photochemical Activation of Protein Expression in Bacterial Cells**

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Small-molecule-inducible gene-expression systems are available in a variety of cell types including bacterial cells, yeast cells, and mammalian cells.[1] They have been used for conditional protein expression in higher organisms ranging from plants to mice.[2] These systems are typically composed of a natural receptor taken from one organism and transferred into a second organism, in conjunction with a natural or unnatural small-molecule ligand that is orthogonal to all endogenous molecules of the organism of interest. Exposure of the organism to the small molecule typically activates gene expression, a process that has found application in the production of recombinant proteins, the programing of biological processes, and the study of gene function. Although this technique enables temporal control over gene function on a minute-to-hour timescale, it does not permit spatial control.

A highly efficient way to simultaneously achieve spatial and temporal control over biological processes is the application of photocaging.[3] The term "caging" defines the installation of a photoremovable group on a biologically active molecule, thus rendering the molecule inactive. Caging groups have been installed on small molecules, oligonucleotides, peptides, and proteins. Irradiation with UV light removes the caging group and restores biological activity. Recently, this approach has been applied to the light activation of small-molecule inducers of protein expression. Both reported systems, that is, the doxycycline and the nuclear hormone (for example, estradiol) conditional-gene-expression systems,[4] are restricted to eukaryotic cells.

Here we report a light-inducible gene-expression system which can be used in bacterial cells, plants, and mammalian organisms. It is based on the lactose (lac) repressor which binds to the *lac* operator (*lac*O), thereby inhibiting RNA polymerase from performing gene transcription.[5,6] In presence of the small-molecule effector isopropyl-β-D-thio-galactoside (IPTG), the repressor is released from the DNA through an allosteric binding event, which results in a conformational change and leads to gene expression (Figure 1).

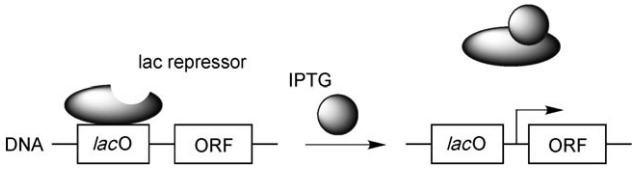

Figure 1. IPTG-induced expression of an open reading frame (ORF) through formation of the IPTG/lac repressor complex.

The crystal structure of the LacI/IPTG complex (Protein Data Bank file no. 1LBH) reveals interactions between the small molecule and the protein in a tight binding pocket, as well as four essential hydrogen bonds between the 4-OH group and Arg197, the 3-OH group and Arg197, and also the 2-OH group and Asp274 and Asn246 (see the Supporting Information).[7] We speculated that disruption of the hydrogen-bond network through the installation of a sterically demanding caging group on IPTG would inhibit the formation of the LacI/IPTG complex and thereby inhibit gene expression. If the installed group is removable through irradiation with UV light, IPTG can be generated in a spatiotemporal manner, thereby enabling spatiotemporal control over protein expression. In order to achieve this, the caged IPTG **1** was synthesized in a single step (78% yield) by the reaction of IPTG with 6-nitropiperonal (see the Experimental Section), which furnished selective dioxolan formation at the 4- and 6-hydroxy groups. This is in accordance with the results of NMR experiments and previous observations in similar reactions.[8] The caged IPTG **1** is nontoxic to bacterial cells and no degradation was observed under physiological conditions. Irradiation of an aqueous solution of **1** (≤ 0.5 mM, $\varepsilon_{365} = 4533\ cm^{-1}M^{-1}$) with nonphotodamaging UV light (handheld UV lamp, 365 nm, $0.5\ W cm^{-2}$) for 5 min leads to quantitative formation of the ester **2**, as a 1:1 mixture of regioisomers (4-OH/6-OH, Scheme 1 shows the 4-OH ester). The half life for the conversion **1**→**2** after irradiation depends on the concentration of **1** and amounts to 11 s at a concentration of 0.1 mM, 5.1 min at 0.5 mM, or 11.8 min at 1.0 mM (see the Supporting Information). This facile photolytic conversion of a 1,3-dioxane is in remarkable contrast to previous observations with bromohydroxycoumarin caged diols.[9] The quantum yield ($\phi = 0.131$) for the photochemical conversion of **1** into **2** has been determined by 3,4-dimethoxynitrobenzene actinometry.[10] The ester **2** is stable in an aqueous solution; however, as observed for other carbohydrate esters, **2** is hydrolyzed to IPTG by the esterases found in a cellular environment ($t_{1/2} = 63$ min ± 2 min, see the Supporting Information). The growth of bacterial cells exposed to 0.5 mM concentrations of **1** and **2** revealed that the compounds do not reduce growth rates, are nontoxic, and are easily taken up by the cells (see the Supporting Information).

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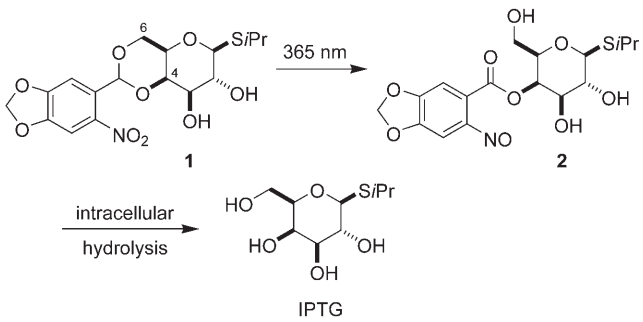

Scheme 1. Light irradiation of caged IPTG **1**, followed by intracellular hydrolysis of the ester **2** to yield IPTG.

Photochemical induction of liquid-culture expression of a β-galactosidase reporter showed a strong dependence of the expression on UV irradiation. It is important to note that this photochemical induction is only possible in cells which express the lac repressor. A pUC19 plasmid containing the *lacZ* gene under the control of the lac operator was utilized to perform a Miller assay in BL21(DE3) cells,[11] thus allowing quantification of the regulation of gene expression through caged IPTG. Cells were grown to an optical density (at 600 nm, OD_{600}) of 0.6, then induced with either IPTG or caged IPTG (to a final concentration of 0.5 mM), and either irradiated or kept in the dark. After 6 h, cells were lysed and treated with *o*-nitrophenyl galactopyranoside and the absorbance of the lysates was measured at 420 and 550 nm (Figure 2). The noninduced control and the cells growing in

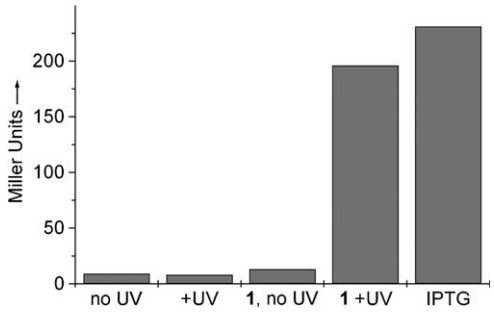

Figure 2. β-Galactosidase (Miller) assay showing that, in the presence of **1**, *lacZ* expression is only observed in cells which have been irradiated with light at 365 nm (5 min, 25 W) or were incubated with IPTG. In the absence of the small-molecule inducer, minimal protein expression is observed. The concentration of the small molecule was 0.5 mM in all cases.

the presence of caged IPTG **1** displayed no β-galactosidase activity, thereby demonstrating that installation of a caging group on the 4- and 6-OH groups completely disrupts binding of IPTG to the lac repressor. Additionally, irradiation of a bacterial culture with UV light in the absence of any small molecule failed to induce β-galactosidase expression. Brief irradiation with nonphotodamaging UV light (365 nm, 15 min, 25 W, hand-held UV lamp) of cells grown in presence of **1** (0.5 mM) led to rapid decaging and levels of protein expression that were comparable to a standard IPTG induction (Figure 2).

An application of the developed light-inducible gene-expression system was demonstrated by bacterial lithography. Here, the synthetic small molecule **1** functions as a light sensor to transform a lawn of bacteria into a biological film. The resolution of such a film has been estimated to be 15 megapixels cm^{-2}; however, this theoretical limit might be diminished through IPTG diffusion.[12] For bacterial lithography, the *lacZ* reporter gene was used to convert colorless 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) into a blue dye. Hence, a negative image is obtained where irradiated regions are dark and nonirradiated regions remain white. The same pUC19 plasmid was employed as before in BL21(DE3) cells. Cells were seeded on agar surfaces pretreated with caged IPTG **1** and X-gal; this was followed by immediate irradiation for only 30 s at 365 nm (25 W). A mask blocking one half of the plate was used to demonstrate spatial control over gene expression. After 12 h of incubation at 37 °C, only cells on the irradiated half of the plate showed a blue color and hence β-galactosidase activity (see the Experimental Section and Figure 3). To obtain a positive

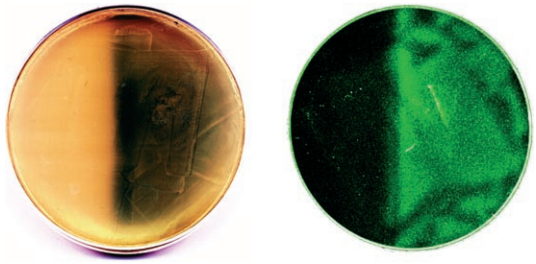

Figure 3. Bacterial lithography with UV irradiation at 365 nm for 30 s while the left half of the Petri dish (∅10 cm) is blocked. Two different reporter genes were employed, namely lacZ (left) and GFP (right), to deliver negative and positive images, respectively.

image, an identical experiment was performed with green fluorescent protein (GFP) as the reporter. Here, *Escherichia coli* cells transformed with a pGFPuv plasmid were seeded on agar plates pretreated with caged IPTG and the plates were irradiated as before and incubated at 37 °C for 12 h. Plates were analyzed for fluorescence on a Storm 480 Phosphor-Imaging system, with the results indicating a spatially restricted expression of GFP (Figure 3) and revealing a positive image. In both cases, spatial control of gene expression is observed, with minimal diffusion of irradiated IPTG. The short exposure time is advantageous over the 12–15 h of light exposure required for previously reported bacterial lithography methods.[12]

In summary, we have achieved light-controlled activation of gene expression in bacterial cells in a spatiotemporal fashion. Due to the widespread application of the lac repressor in biotechnology, broad utilization of the developed methodology is expected. Moreover, since the lac repressor is also functional in plant cells, mammalian cells, and higher organisms,[5] this approach has the potential to provide a general solution for temporal and spatial control over gene

function in more complex biological environments. Importantly, it could allow for a photochemical regulation of bacterial quorum sensing,[13] thereby providing detailed information on the mechanism of biofilm formation through cell-to-cell communication.[14] Additional applications range from the creation of biological materials with certain spatial patterns to the study of genetic circuits[15] in developmental model organisms.

Experimental Section

Synthesis of **1**: Isopropyl-β-D-thio-galactoside (50 mg, 0.18 mmol) was dissolved in dimethylsulfoxide (DMSO; 0.5 mL) at 0 °C. 6-Nitropiperonal (103 mg, 0.53 mmol) and concentrated H_2SO_4 (0.2 mL) were added, and the reaction was allowed to warm to room temperature. After 24 h, the reaction was quenched with water (0.5 mL), the layers were separated, and the organic layer was extracted with ethyl acetate (3 × 2 mL). The combined organic layers were dried and concentrated, and the residue was subjected to column chromatography on SiO_2 (EtOAc/hexanes 5:1) to yield a yellow solid (56 mg, 0.13 mmol, 78%): ^{1}H NMR (300 MHz; $CDCl_3$): $\delta = 7.40$ (s, 1H), 7.33 (s, 1H), 6.16 (s, 1H), 6.10 (s, 2H), 4.40 (d, $^3J(H,H) = 9.3$ Hz, 1H), 4.29 (m, 2H), 4.08 (m, 2H), 3.70 (m, 2H), 3.50 (m, 1H), 3.25 (septet, $^3J(H,H) = 6.9$ Hz, 1H), 1.39 ppm (d, $^3J(H,H) = 6.9$ Hz, 3H), 1.37 ppm (d, $^3J(H,H) = 6.9$ Hz, 3H); ^{13}C NMR (75 MHz; $CDCl_3$): $\delta =$ 151.79, 148.30, 128.94, 107.77, 105.43, 103.32, 96.89, 85.81, 76.25, 74.04, 70.39, 70.20, 69.91, 35.64, 24.53, 24.34 ppm; HRMS (FAB): *m*/z calcd. for $C_{17}H_{21}NO_9S$: 416.1015, found: 415.0984.

Spatial control of protein expression: Ampicillin-resistant constructs pUC19 or pGFPuv with the *lacZ* or the *gfp* gene, respectively, under the control of the lac repressor in BL21(DE) cells, were grown to log phase for 12 h at 37 °C in Luria–Bertani broth containing 20 μg mL^{-1} of ampicillin (LB/Amp). Bacteria (100 μL, $OD_{600} = 1.1$) were then spread as lawns on LB/Amp agar plates (∅10 cm), which were pretreated with caged IPTG (10 mM, 40 μL) and X-gal (2% in *N*,*N*-dimethylformamide (DMF), 40 μL) in the case of lacZ expression. One half of the plate was covered with a mask, and the plate was irradiated for 30 seconds at 365 nm. After the brief irradiation, the bacteria were grown for 12 h at 37 °C, and the plates were imaged.

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