J|A|C|S COMMUNICATIONS

Published on Web 10/07/2009

A Photocontrolled Molecular Switch Regulates Paralysis in a Living Organism

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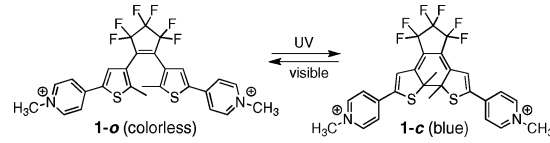
The use of light to trigger changes in molecular systems has great importance in biochemistry and medicine.[1] Systems based on the reversible photoreactions of diarylethenes have advanced the area of 'smart' materials, and their potential use as components of molecular electronics, optical memory, and variable-transmission filters has been well documented.[2] Their appeal is based on the fact that light can be tuned to reversibly and specifically trigger optical and electronic changes such as color, emission, and refractive index in materials that contain the versatile molecular architecture.[3] However, despite the large body of literature describing the use of diarylethene photoswitches in Materials Science technologies, their ability to reversibly control chemical and biochemical reactivity has only recently been demonstrated.[4] They have not been used *in vivo* as drug delivery vehicles or to unmask therapeutic agents,[5] where issues such as whether the photoresponsive systems can be absorbed by a living organism, if they retain their reversible photoactivity, and if the two forms of the molecular switch have unique effects on the function of the living organism must be considered.

Here we demonstrate for the first time that the light-induced reactions of a photoresponsive dithienylethene can be reversibly triggered in a living organism and that the photoswitch induces paralysis in *Caenorhabditis elegans* only when specific wavelengths of light convert it to one of its structural forms.

The photoswitch used in the *in vivo* studies described in this communication is bis(pyridinium) DTE **1** (Scheme 1), which undergoes cyclization from a colorless, ring-open isomer (**1-*o***) to a colored, ring-closed isomer (**1-*c***) when exposed to UV light (300−400 nm). The reverse, ring-opening process is triggered with visible light of wavelengths greater than 490 nm. The specific photoresponsive architecture has many appealing features. (1) Neither isomer spontaneously converts to the other at ambient temperatures (25−40 °C) in the absence of light. (2) The photoreactions tend to proceed with a high degree of efficiency and with minimal degradation. (3) The charged nature of **1-*o*** renders it soluble in an aqueous solution that can be orally administered. (4) The compound emits light, which allows its uptake in living organisms to be easily monitored using fluorescence microscopy. (5) Most importantly, the two isomers of the photoswitch have significantly different abilities to act as electron-accepting species. In the medium used to administer the compound to the nematodes (10% DMSO in pH 7.0 phosphate buffer), isomer **1-*c*** is easily reduced (at −350 mV) while **1-*o*** is reduced along with the solvent at potentials more negative than −1.0 V suggesting that only the ring-closed isomer will interfere with the metabolic pathway required for energy production in *C. elegans*.[6] Compounds with reduction potentials ranging from −230 to −720 mV in phosphate buffer (pH 7.0) are known to have this effect, while compounds with reduction potentials similar to that for the ring-open isomer (**1-*o***) do not.[7]

Scheme 1[8]



Caenorhabditis elegans is the model organism of choice for the *in vivo* photoswitching experiments. It has the advantage of being a simple multicellular eukaryotic organism that has been studied in great detail. It is also one of the simplest organisms with a nervous system, having 302 neurons, which explains why it has been extensively used as a model for neuroactivity and metabolism.[9] Moreover, its transparent nature makes it an ideal candidate for monitoring color changes *in vivo*.

The *in vivo* effect **1** has on *C. elegans* nematodes was demonstrated by feeding them the compound and toggling the photoswitch back-and-forth between its two isomers using UV and visible light. This was achieved by incubating 3−4 day old wild-type N2 hermaphrodite *C. elegans* with the photoswitch in either of its forms (**1-*o*** and **1-*c***) at room temperature in aqueous phosphate buffer (12 mM) containing 10% v/v DMSO. When aliquot amounts of the *C. elegans* were withdrawn from each sample within the first 10 min and after 60 min of incubation, washed twice with phosphate buffer, and imaged using fluorescence microscopy, significant oral uptake of both forms of the photoswitch is evident as demonstrated by the observable fluorescence emitting from the body of the nematodes fed the photoswitch compared to samples fed only DMSO (Figure 1a vs 1b and 1c). This clearly illustrates that the organisms have orally consumed the photoswitch, an observation that has been confirmed by multiple experiments.[10]

Importantly, the photoresponsive compound retains its photoactivity (**1-*o*** ⇆ **1-*c***) even when inside the body of the nematodes as confirmed by light microscopy (Figure 1d and 1e). Exposing the colorless nematodes, which were fed the ring-open form of the compound, to 365-nm light for 2 min resulted in an immediate appearance of the blue color that is typical for this specific photoswitch as it undergoes the ring-closing reaction. The fact that the color is apparent throughout the body illustrates that the photoswitch has either diffused or has been transported by the organisms. Visible light of wavelengths greater than 490 nm triggered the reverse reaction, regenerating the ring-open isomer, and the nematodes became colorless. This coloration−decoloration cycle can be repeated several (at least three) times. Nematodes that were fed the ring-closed form of the compound (**1-*c***) had an apparent blue color and could be transferred to being colorless upon exposure to visible light of wavelengths greater than 490 nm for 20 min. Nematodes incubated with DMSO alone did not react to UV light at 365 nm and appeared viable.

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10.1021/ja903070u CCC: $40.75

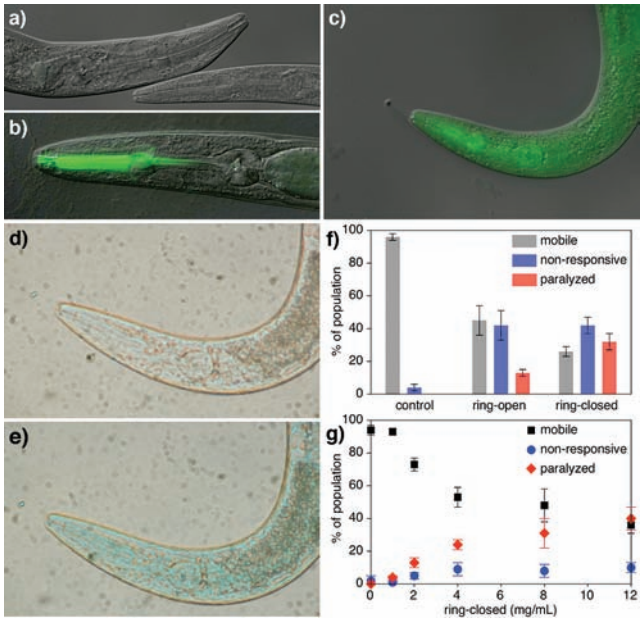

Figure 1. Fluorescence microscopy images of *C. elegans* incubated with 10% DMSO (a) and photoswitch **1-*c*** (12 mM) within the first 10 min (b) and after 60 min (c).[11] Similar results were achieved with nematodes incubated with **1-*o***. Optical microscopy shows the photoswitch being interconverted between colorless **1-*o*** (d) and blue **1-*c*** (e) with light of wavelengths greater than 490 nm and UV light (365 nm) for 20 and 2 min, respectively. (f) The number of mobile, nonresponsive, and paralyzed nematodes for samples that have been treated with the ring-open or the ring-closed form of the photoswitch compared to controls after 60 min incubation and (g) for samples exposed to varying amounts of **1-*c***. Error bars represent the Standard Error of Mean.

The presence of **1** has a remarkable effect on the mobility of the nematodes depending on which isomer is present. Control nematodes (those fed only DMSO) plated on agar containing *Escherichia coli* OP50 as a food source appear mobile and lively, moving by chemotaxis toward the bacteria where they can feed and lay eggs (676 worms). However, nematodes fed **1-*c*** (12 mM) for 60 min (313 worms) appeared paralyzed and immobile (Figure 1f).[12] The dose−response curve of using **1-*c*** at 0, 2, 4, 8, and 12 mM (average of 250 worms per experiment) showed that 12 mM is the most effective concentration (Figure 1g).

The ring-open form (**1-*o***) appears to induce significantly less paralysis in the nematodes (260 worms), albeit, both forms are eventually toxic to the organisms. The paralysis induced by **1-*c*** can be reversed by exposing the nematodes to visible light (20 to 25 min) at the wavelengths required to convert **1-*c*** to **1-*o***, by which time the nematodes regain their mobility and appear as if they had never been paralyzed. The paralysis can be turned 'on' and 'off' by cycling the photoswitch between its ring-open and ring-closed form within the same nematodes by alternating 365-nm light (for 2 min; paralysis is observed 10 to 15 min later) and visible light (>490 nm; for 20 to 25 min).[13] The paralysis is clearly an effect of the presence of the photoswitch, as the control nematodes (676 worms) not treated with the compound appear unaffected by exposure to either UV or visible light.

The differing response of the nematodes to the presence of the photoswitch may be due to the extent the organisms process the compound. Initially, the uptake is mainly in the mouth region. After 60 min, some nematodes appeared to process the compound further throughout their bodies, which may lead to 'nonresponsive' behavior. Most of these nematodes were actually alive as observed by the pumping of their pharyngeal bulbs when viewed under the microscope. In other worms, the compound was less distributed throughout the bodies and was concentrated mainly in the head region, which may result in the reversible paralysis. In the extreme cases, some nematodes showed either no uptake of the compound or localization of it only in the head region resulting in minimal effects.

The unique ability of the photoresponsive dithienylethene to reversibly isomerize between biologically active (**1-*c***) and inactive (**1-*o***) forms when irradiated with different wavelengths of light proves that 'on−off' control of biological functions can be achieved. This example illustrates how photoswitches offer great potential for advancing biomedical technologies such as photodynamic therapy as alternative, noninvasive surgical treatments. In these studies we have demonstrated that an appropriately chosen photoswitch can be taken up by a living organism and still retain its photoswitching behavior *in vivo*. In this particular example, the unique electron-accepting ability of one of the photoisomers to induce paralysis in *C. elegans* is likely a result of interruption of the metabolic electronic pathway and the resulting loss of energy production. Future work will focus on determining the validity of this claim.

Acknowledgment. This research was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canada Research Chairs Program and Simon Fraser University (SFU) through the Community Trust Endowment Fund. This work made use of 4D LABS shared facilities supported by the Canada Foundation for Innovation (CFI), British Columbia Knowledge Development Fund (BCKDF), and Simon Fraser University.

Supporting Information Available: Methods and video file of reversible nematode paralysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The counterions are initially PF_6^- but likely exchange for PO_4^{2-} when the compound is dissolved in the buffer used for in vivo experiments.
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(10) Each experiment was performed multiple times on different batches of nemotodes: control, 8 studies, 16 plates, 676 worms; **1-*c***, 3 studies, 10 plates, 313 worms; **1-*o***, 2 studies, 6 plates, 260 worms.
(11) Worms showed differing amounts of uptake and processing of the dye within the first 60 min. See Supporting Information for details.
(12) In these studies, three different states of mobility were observed (Figure 1f). After being plated, the nematodes were gently prodded with a small metal wire. Any nematodes that moved rapidly were considered 'mobile', those that showed small movements in parts of their bodies but lacked mobility were considered 'paralyzed', and those that showed no response were considered 'nonresponsive'. In many cases, 'paralyzed' nematodes appeared 'nonresponsive' until prodded.
(13) See online Supporting Information for details.

JA903070U