Photoresponsive Molecules

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Turning "On" and "Off" a Pyridoxal 5′-Phosphate Mimic Using Light**

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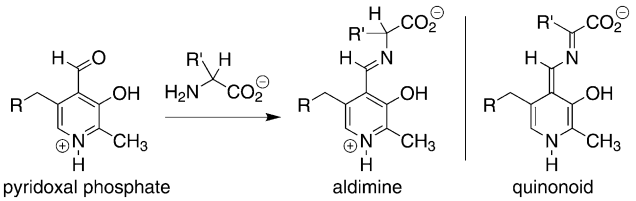
The use of light to reversibly change the structure of biologically relevant molecules and turn "on" and "off" important biochemical functions "on-command" offers the biomedical end-user a non-invasive, rapid, reversible, spatial and temporal tool for research and therapy. Applying this control strategy to macromolecules such as oligonucleotides and proteins is challenging because their large size and complexity makes it difficult to target a particular area on the macromolecules for modification, although several impressive examples have been reported.[1–14] The alternative is the use of photoresponsive small molecules that play an intimate role in biochemical processes, as either cofactors or inhibitors. Not only would these molecular systems be easier to photoactivate and deactivate than their enzyme partners, they would also provide a more "universal" method to regulate biological function because the same cofactor can be involved in more than one operation.

We have recently described how two different colors of light can be used to convert a small molecule between two isomeric forms differing by an order of magnitude in their ability to act as an inhibitor for human carbonic anhydrase.[15] While regulating inhibitors is appealing,[16–18] applying the same strategy to enzyme cofactors would provide control over a more diverse set of biochemical systems. Cofactors have all the earmarks of a suitable photoresponsive target. They tend to be small in size, structurally simple and easy to modify and study, while still allowing for ultimate control over the enzyme activity. Here, we take a first logical step by using a well-known enzyme cofactor as the inspiration in our design of a proof-of-concept demonstration. We show how our biomimetic system acts as a photoswitchable catalyst for a biochemical reaction.[19]

The biologically active form of vitamin B_6, pyridoxal 5′-phosphate (PLP), is a versatile enzyme cofactor responsible for amino acid metabolism in all organisms from bacteria to humans.[20] Its participation in a diverse range of enzymatic reactions including transamination, racemization, decarboxylation, and numerous elimination and replacement processes makes it unrivalled.[21] It is a particularly inspiring cofactor for our proof-of-concept design because it can catalyze many processes without the presence of an enzyme.[22,23] It is also the role-model target of the studies described in this report.

The structural features responsible for the action of PLP are the aldehyde and pyridinium functional groups, which are electronically connected to each other through bonds (Scheme 1). This intimate connectivity allows any molecule attached to the aldehyde to "sense" the electron withdrawing nature of the positively charged heterocycle. An example of this is the aldimine generated when an amino acid condenses with PLP (Scheme 1) and it is this Schiff base that is responsible for the enormous range of reactions the cofactor catalyzes.[24] The pyridinium group in the aldimine makes the amino acid's α-hydrogen more acidic by stabilizing the negative charge in the conjugate base through contribution from a quinonoid structure (Scheme 1).[25,26] The fate of this intermediate is varied and is responsible for the numerous biochemical outcomes when PLP is involved.



Scheme 1. Reaction of an amino acid with PLP showing the aldimine produced (R = OPO_3^{2-}; R′ = side chain). The quinonoid structure formed after removal of the amino acid's α-hydrogen is also shown.

We have developed a photoresponsive PLP cofactor mimic whose catalytic activity can be reversibly switched "on" and "off" when desired by irradiating it with UV and visible light, respectively. We achieved this goal by taking advantage of the photoswitchable dithienylethene (DTE) architecture (Scheme 2), which undergo ring-closing and ring-opening reactions when exposed to UV and visible light, respectively.[27–29] The ring-open form of our system (**1*o***) lacks extensive through-bond communication between its two "arms" (the ones bearing the pyridinium and aldehyde functional groups). They are effectively electronically insulated from each other and the cofactor mimic is "inactive". Ring-closing with UV light generates a linearly conjugated π-backbone (shown in bold in structure **1*c*** in the scheme) that connects the two functional groups and the participation of a quinonoid intermediate can be expected in the aldimine produced when isomer **1*c*** reacts with amino acids (shown on the right of Scheme 2). Only the ring-closed isomer is "active" and mimics PLP.

Our photoresponsive catalyst (**1*o***) was prepared as outlined in Scheme 3. (Synthetic procedures and characterization

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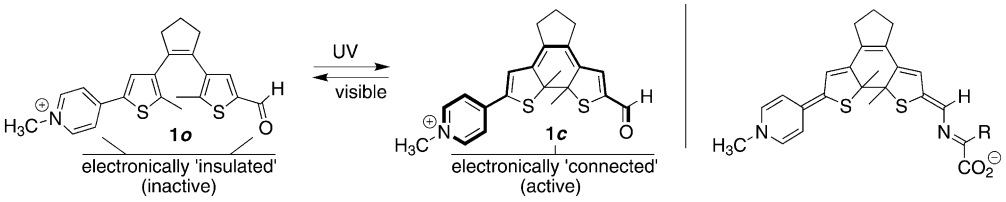
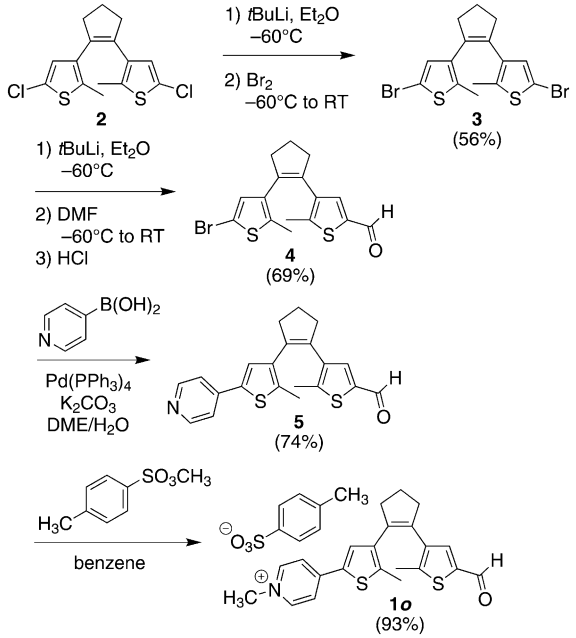


Scheme 2. UV and visible light toggles the dithienylethene structure between its "inactive" ring-open (**1*o***) and "active" ring-closed (**1*c***) forms and dictates if the pyridinium and aldehyde are insulated or connected to each other. The structure on the right is the quinonoid that would be generated after deprotonating the α-hydrogen of the aldimine generated from reaction of compound **1*c*** and an amino acid.



Scheme 3. Synthesis of the photoresponsive catalyst.

of all compounds are provided in the Supporting Information.) Compound (**1*o***) undergoes light stimulated ring-closing as is expected for dithienylethenes. This reaction is illustrated by the changes in the UV/Vis absorption spectra when an aqueous acetic acid solution of the compound is irradiated with 365 nm light (Figure 1) and is accompanied by a change in color of the solution from pale yellow to deep blue. As is typical for dithienylethene photoswitches, there is an immediate reduction in the intensity of the high-energy bands and the appearance of a new set of bands in the visible region of the spectrum (centered at 635 nm) that correspond to the ring-closed isomer (**1*c***). ^{1}H NMR spectroscopy confirmed the conversion (**1*o*** → **1*c***) and revealed that the photostationary state contains 97% of the ring-closed isomer, the remainder consisting of the ring-open isomer (**1*o***). Visible light of wavelengths greater than 490 nm converted the solution back to pale yellow and regenerated the ring-open isomer (**1*o***).

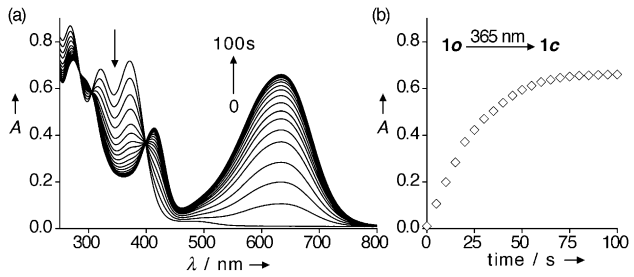


Figure 1. a) Changes in the UV/Vis absorption spectrum when a CH_3CO_2H/H_2O (9:1 v/v) solution (5×10^{-5} M) of **1*o*** is irradiated with 365 nm light. b) The growth of the band at 635 nm corresponding to the ring-closed isomer (**1*c***), which is present in 97% in the photostationary state according to ^{1}H NMR spectroscopy.

The ability of our photoresponsive PLP mimic to act as a controllable catalyst was demonstrated using hydrogen–deuterium exchange experiments and ^{1}H NMR spectroscopy on solutions of optically pure L-alanine (Figure 2). This is an accepted method to monitor the catalytic racemization of amino acids since racemization is expected to occur through the planar, achiral sp^2 hybridized quinonoid intermediate. The rates of hydrogen–deuterium exchange and racemization in a series of amino acid derivatives have been shown to be nearly equal.[30]

Our experiments were performed by dissolving L-alanine (1 molar equivalent) and a catalytic amount of either **1*o*** or **1*c*** (0.2 molar equivalents) in a 1:9 (v/v) mixture of D_2O and [D_4]acetic acid at 40 °C (± 2 °C) in a sealed NMR tube. The solution of the ring-closed isomer was prepared by irradiating **1*o*** with 365 nm light until no changes were observed in the ^{1}H NMR spectrum (typically 120 min at this high concentration). The partial ^{1}H NMR spectrum in Figure 2b and c shows the set of peaks corresponding to the α-hydrogen of L-alanine. It is clear from this figure that the intensity of the peaks is greatly reduced when the ring-closed isomer (**1*c***) is present but the intensity remains the same in the case of the ring-open isomer (**1*o***). Only the former isomer can induce deprotonation of the amino acid. This exchange process presumably occurs through the aldimine intermediate, although under these conditions this intermediate was not observed in the ^{1}H NMR spectrum. Another indication that only the ring-closed isomer catalyzes the exchange process is the conversion of the peak corresponding to the CH_3 groups of L-alanine from a doublet to a singlet as hydrogen–deuterium exchange proceeds Figure 2d. This spectral change was not observed when the ring-open isomer (**1*o***) was used.

After the hydrogen–deuterium exchange catalyzed by the ring-closed isomer (**1*c***) was nearly complete (95% as confirmed by ^{1}H NMR spectroscopy), alanine was isolated from the reaction mixture. In the partial ^{1}H NMR spectrum (D_2O) of this sample (Figure 2e bottom), the singlet at 1.21 ppm corresponds to the CH_3 group of the amino acid after hydrogen–deuterium exchange. The smaller peak appearing at 1.22 ppm corresponds to the residual non-

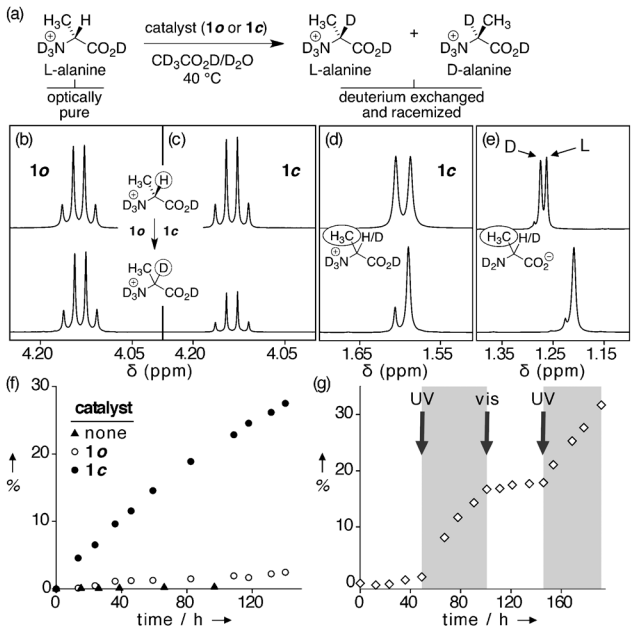


Figure 2. a) The racemization of L-alanine can be indirectly assessed by monitoring the exchange of the α-hydrogen by a deuterium in the presence of **1*o*** or **1*c***. The ring-closed isomer was prepared by irradiating (**1*o***) with 365 nm light for 120 min. b–d) Partial ^{1}H NMR spectra of CD_3CO_2D/D_2O (9:1 v/v) solutions of L-alanine (0.1 M) containing 0.2 equiv of b) **1*o*** and c,d) **1*c*** at 40 °C (±2 °C). In each case, the top spectrum corresponds to the solution at the beginning of the experiment and the bottom spectrum is after 20 days. e) The alanine isolated after reaction with **1c** (for an additional 90 h at 55 °C) with (top) and without (bottom) 0.1 molar equivalents of sodium [(*R*)-1,2-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetato]samarate(III). Both samples were basified to pH 10–11 with NaOD (40% w/w in D_2O) to ensure binding to the chiral shift reagent. f) Percent hydrogen–deuterium exchange of the L-alanine solutions without catalyst (▲), in the presence of 0.2 equiv of catalyst, **1*o*** (○) or **1*c*** (●) over the first 140 h. g) Changes in the percent hydrogen–deuterium exchange of CD_3CO_2D/D_2O (9:1 v/v) solution of L-alanine (0.2 M) containing 0.2 equiv of **1*o*** in response to alternating cycles of irradiation with UV and visible light. The timescale referred to on the *x*-axis is the total reaction time at 40 °C and does not include ^{1}H NMR acquisition time and irradiation times. The ^{1}H NMR measurements and sample irradiations were conducted at room temperature where the rate of hydrogen–deuterium exchange is insignificant.

deuterated alanine, which is actually an overlapping doublet. The addition of an NMR chiral shift reagent ([(*R*)-1,2-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetato] samarate(III)) to a solution of the isolated amino acid in D_2O produces two well-resolved diastereomeric complexes with unique chemical shifts in the ^{1}H NMR spectrum as shown in (Figure 2e top). Each singlet can be assigned to the CH_3 group of D-alanine and L-alanine, and their approximately equal intensity confirms that racemization has occurred.

The rate of deuterium exchange was monitored in situ by recording the decrease in integration under the curve of the peaks in the spectrum corresponding to the α-hydrogen of the amino acid (4.13 ppm) as it is converted to α-deuterium. A plot of percentage of hydrogen–deuterium exchange over time (Figure 2f) emphasizes the minimal racemization of L-alanine when **1*o*** is present (less than 3% exchange observed after a total of 140 h at 40 °C). On the other hand, within this same time period the ring-closed isomer (**1*c***) catalyzes the exchange to nearly 30%, and when allowed to react for an additional 90 h at 55 °C we achieve nearly complete (95%) hydrogen–deuterium exchange. With no catalyst present there is essentially 0% exchange showing that the racemization of the amino acid substrate on its own is negligible.

We could also reversibly turn "on" and "off" our catalyst by using UV and visible light (Figure 2g). When a solution of L-alanine is treated with the ring-open isomer (**1*o***) under the same conditions as described previously, as expected there is little observable hydrogen–deuterium exchange. After exposure of the sample to 365 nm light, which generates the ring-closed isomer (**1*c***), we observed immediate exchange. Converting the catalyst back to its ring-open form with visible light reduces the exchange to its originally low rate. The rate can be increased again by using UV light.

The relative rates of deuterium exchange reflect the differences in electronic properties of the ring-open and ring-closed isomers of our PLP mimic and demonstrate the success of our concept. The higher rate of exchange when **1*c*** is present supports the design strategy wherein the pyridinium is able to communicate through the DTE backbone with the aldehyde only in this isomeric state. This provides evidence for a dramatic increase in activity when in the ring-closed form and potentially offers new means to control enzymatic processes by modifying appropriate cofactors with photoresponsive components. Although rates of racemization are low, the system demonstrated in our studies is the first example of photoswitchable PLP mimic that can act as a catalyst.

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