Light-Regulated Enzyme Activity

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Regulation of Human Carbonic Anhydrase I (hCAI) Activity by Using a Photochromic Inhibitor**

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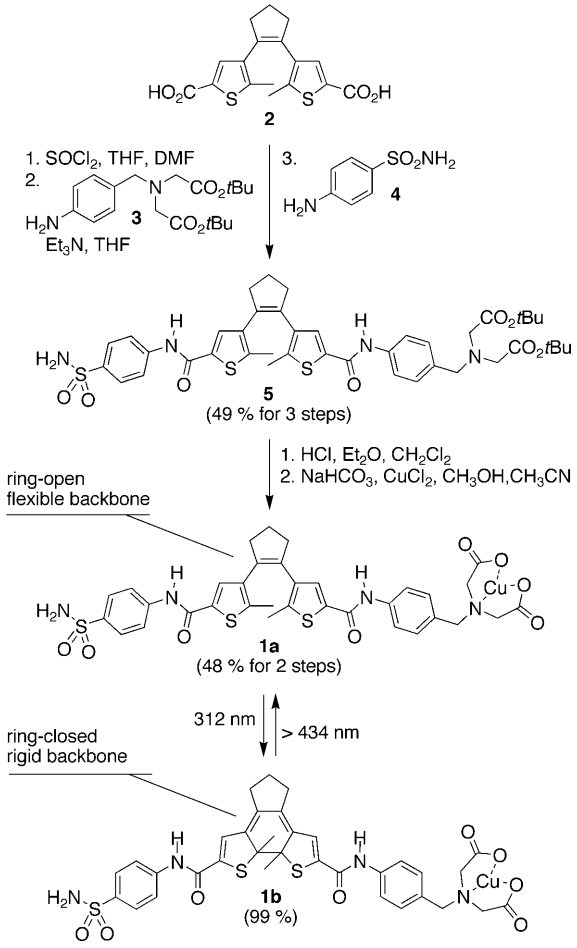
The regulation of enzyme activity is crucial for the metabolism of every organism. In biology, enzymatic control is typically achieved through the use of allostery[1] or by covalently modifying the enzyme (by phosphorylation or dephosphorylation, for example).[2] Some of the original attempts to artificially influence the activity of enzymes rely on chemical modifications of the enzyme structure,[3–6] an approach that is limited by the fact that the regulation is not reversible. The use of light as a stimulus offers a heightened level of control, and photoresponsive compounds would provide the reversibility needed for practical use. Existing examples of systems that take advantage of the beneficial properties of light include those that use azobenzene-based enzyme inhibitors[7] or use thiophenfulgide derivatives covalently linked to the enzyme.[8] Photoinducing changes in the environment around the enzyme has also been used as a regulation mechanism by influencing the permeability of a photoisomerizable polymer containing the enzyme for the substrate,[8] by controlling the conformation of a specific domain of the enzyme with surfactants,[9] and by changing the conditions of the medium (pH or viscosity, for example).[10]

Controlling the activity of carbonic anhydrase is of special interest as it is an enzyme central to both cellular transport and metabolic processes. It can be found in virtually every tissue and cell type, in many subcellular organelles, and in organisms ranging from unicellular cyanbacteria to mammals.[11] Recently, an azobenzene-based biolabel was used to photomodulate the activity of carbonic anhydrase (by about twofold).[12] Although controlling enzyme activity with azobenzene derivatives is an elegant concept, the thermal reversibility that plagues these particular photoresponsive compounds significantly limits their use in practical applications. On the other hand, compounds constructed from the 1,2-dithienylethene (DTE) scaffold represent a significant improvement over most other photoresponsive structures, primarily because they undergo thermally irreversible photochemical ring-closing and ring-opening reactions (see, for example, the substructures **1a**⇄**1b** in Scheme 1; **a**: open, **b**: closed).[13]



Scheme 1. Synthesis and the reversible photochemical ring-closing reaction of DTE inhibitor **1a**.

Herein, we describe how this versatile photoresponsive structure can be used to reversibly control the activity of carbonic anhydrase by decorating the DTE architecture with sulfonamide and copper(II) iminodiacetate {Cu(ida)} moieties. These two moieties were chosen in light of a recent report by Mallik et al. on a significant increase in the activity of the weak enzyme inhibitor sulfanilamide (4-aminobenzenesulfonamide, **4**) upon covalently linking it to a {Cu(ida)} complex.[14]

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While the sulfonamide group in **1a** acts as the inhibitor, the role of the {Cu(ida)} component is to reversibly coordinate to the imidazole side chains of the histidine residues exposed on the protein surface close to the Zn^{II} active site of the enzyme and help dock the sulfonamide inhibitor group into the catalytic center. Given the fact that the activity of any two-pronged enzyme inhibitor is directly dependent on the distance and relative orientation of the two groups (in this case, {Cu(ida)} and the sulfonamide) and the fact that the DTE architecture can be toggled between a flexible, ring-open (**1a**) and rigid, ring-closed (**1b**) isomer,[15] we designed compound **1** to reversibly photoregulate enzyme activity without having to resort to chemical modifications or changes in the natural environment of the enzyme. The synthesis of compound **1a**[16] started with the stepwise coupling of the acid chloride of cyclopentene **2**[17] with diester **3** and sulfanilamide (**4**; Scheme 1). After removal of the two *tert*-butyl groups with acid, treatment with $CuCl_2$ under basic conditions afforded **1a** in good yield.

Irradiating an aqueous solution of **1a** (5% DMSO, tris(hydroxymethylamino)methane (Tris) sulfate buffer, 20 mM, pH 8.3 at 25 °C) with 312 nm light[18] resulted in the immediate changes in the UV/Vis absorption spectra that are typical for photoresponsive DTE derivatives (Figure 1). The

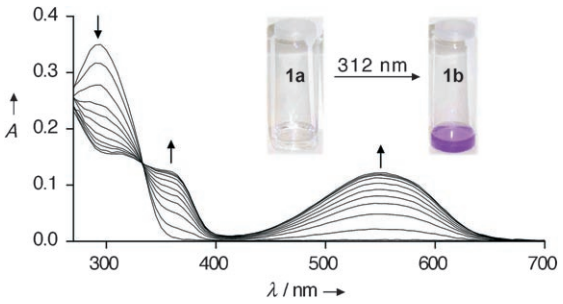


Figure 1. Changes in the UV/Vis absorption spectra of an aqueous solution of **1a** (1.08×10^{-5} M) in DMSO (5% v/v) and Tris sulfate buffer (20 mM, pH 8.3) when irradiated with 312 nm light. Irradiation periods are 0, 3, 5, 8, 10, 14, 17, 20, and 34 s. The inset illustrates the change in color of the solution from colorless to purple as the ring-closed isomer is generated.

high-energy band ($\lambda_{max}=290$ nm) decreases in intensity and an absorption band in the visible spectral region ($\lambda_{max}=$ 545 nm) appears as the solution changes from colorless to purple because of the formation of the ring-closed isomer **1b** (a smaller band at $\lambda_{max}\approx360$ nm also appears). These spectral changes are complete after irradiation for 34 s (at a concentration of 1.08×10^{-5} M), and a photostationary state containing at least 99% of the ring-closed isomer is generated according to HPLC analysis of the reaction mixture. This effective photoconversion attests to the versatility of the dithienylethene backbone as a photoresponsive architecture on which to build practical devices. The large amount of **1b** in the photo-generated mixture is highly beneficial, and a lower amount would make the differences in enzyme inhibition significantly less pronounced. The solution containing the ring-closed isomer is very stable at room temperature as long as it is kept in the dark, and the colored state did not revert to its colorless form even after six months. Irradiation of the colored solution with visible light ($\lambda>420$ nm) converts the ring-closed isomer back into **1a** and regenerates the original absorption spectrum. This ring-closing/ring-opening cycle can be repeated at least seven times without any sign of degradation.[16]

The human carbonic anhydrase I (hCAI)-catalyzed hydration of carbon dioxide [Eq. (1)] is a convenient probe

$$CO_2 + H_2O \xrightarrow{hCAI} HCO_3^- + H^+ \qquad (1)$$

that can be used to investigate the inhibitory effect of the photoresponsive DTE compound in its ring-open (**1a**) and ring-closed (**1b**) states.[16] The known inhibitor, sulfanilamide (**4**),[19] and photoresponsive compounds **6–8** provide excellent controls for comparison. All results are presented in Figure 2 and Table 1.

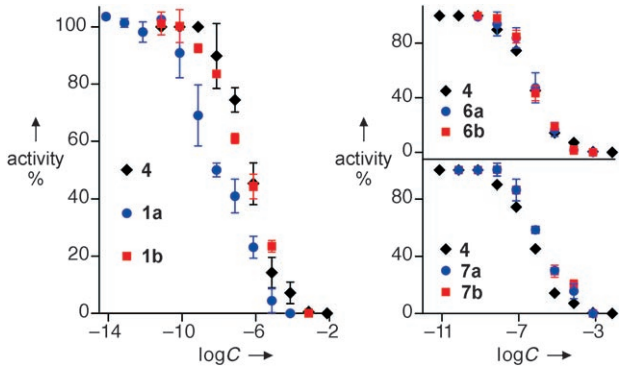
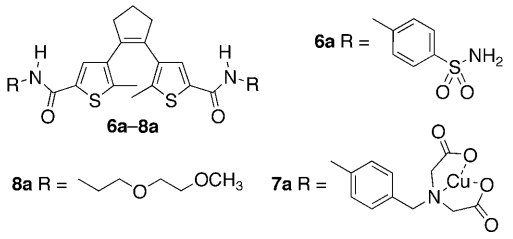


Figure 2. Change in the activity (%) of hCAI when the concentrations of compounds **1**, **4**, **6**, and **7** in their ring-open and ring-closed forms are varied.[16] The data were obtained in an enzymatic assay that monitored the reaction of carbon dioxide and water to generate hydrogen carbonate [Eq. (1)].[22]



Sulfanilamide (**4**) has an IC_{50} value of 0.46 μM, which is in good agreement with that reported in the literature.[20] The inhibition effect of the ring-closed DTE isomer **1b** is comparable ($IC_{50}=0.4$ μM). The similarity of the inhibitor strength of **4** and **1b** suggests that only the sulfonamide component interacts with the active site of the enzyme in the latter compound. This is likely because the planar and rigid backbone in **1b** prevent the simultaneous binding of the {Cu(ida)} and sulfonamide components, and will be elaborated on later in this communication.

Table 1: IC_{50} values and K_i binding affinities of compounds **1**, **4**, and **6–8** in their ring-open and ring-closed forms.

Inhibitor	IC_{50} [μM]		K_i [μM][a]	
	ring-open	ring-closed	ring-open	ring-closed
4	0.46 ± 0.01		0.29 ± 0.007	
1	0.008 ± 0.0003	0.40 ± 0.005	0.005 ± 0.0002	0.30 ± 0.003
6	0.53 ± 0.007	0.57 ± 0.01	0.34 ± 0.005	0.35 ± 0.008
7	1.55 ± 0.8	1.46 ± 0.15	1.16 ± 0.05	1.00 ± 0.01
8	–	–	–	–

[a] The values of K_i were obtained using the Cheng–Prusoff equation.[16]

On the other hand, the ring-open counterpart (**1a**) inhibits the enzyme much more significantly, and its inhibition activity is two orders of magnitude higher ($IC_{50} = 8$ nM) than that of **4** and **1b**. This increase can be attributed to the structural flexibility of **1a**, which allows both recognition components to bind to the enzyme and leads to a higher overall binding affinity. The photoresponsive bis(sulfonamide) **6** shows similar inhibition as sulfanilamide **4**, and no difference between the activity of the ring-open ($IC_{50} = 0.53$ μM) and ring-closed isomers ($IC_{50} = 0.57$ μM) can be observed.[21] In the case of the photoresponsive bis(iminodiacetate) **7**, the IC_{50} value is lower than that of sulfanilamide **4** and but once again, no significant difference between the ring-open ($IC_{50} = 1.55$ μM) and the ring-closed isomers ($IC_{50} = 1.46$ μM) is measured. The photoresponsive bis(ethyleneglycol) **8** was synthesized to investigate whether the dithienylethene unit itself has an influence on the enzyme activity. This compound shows no inhibition in the hCAI-catalyzed hydration of carbon dioxide. All observed changes in the enzyme's activity can, therefore, be ascribed to the synergistic roles the sulfonamide and the {Cu(ida)} groups play as well as to their relative spatial orientation to each other. The binding affinities (K_i) of all compounds show similar trends (Table 1). The exception is ring-open isomer **1a**, which more effectively binds to the enzyme ($K_i = 0.005$ μM for **1a** as compared to ≈0.29–1.16 μM for **1b**, **4**, **6a**, **6b**, **7a**, and **7b**). The reversible DTE ring-closing and ring-opening cycle, converting **1a** into **1b** and back, is also possible in the presence of the enzyme.[16]

As alluded to throughout this communication, we can explain the differences in inhibition and binding affinity of the two photoisomers of **1** by comparing the differences in their conformational flexibility. The flexible ring-open form **1a** was designed to allow the simultaneous docking of the sulfonamide and the {Cu(ida)} components onto the enzyme surface. This is possible because of the free rotation around the C–C single bonds joining the two thiophene heterocycles to the central cyclopentene ring, which allows the inhibitor to adopt a geometry appropriate for bivalent binding only when in its ring-open form. The structure of the enzyme active site (containing both sulfonamide and {Cu(ida)} components; Figure 3) clearly reveals the need for geometric adaptation. The distance between the two binding components (ca. 10 Å) and the way they project in space can only be satisfied by **1a**.

Although the distance between the sulfonamide and {Cu(ida)} components does not change when the antiparallel conformation of **1a** is converted into its ring-closed counterpart (Figure 3), it is the parallel conformation of **1a** that is the likely candidate for bivalent binding to the enzyme. The planar, rigid backbone found in the ring-closed isomer **1b** forces the two components away from each other in a nonproductive manner, allowing only one of the components to bind to the enzyme at a time. This reduces binding and inhibition.

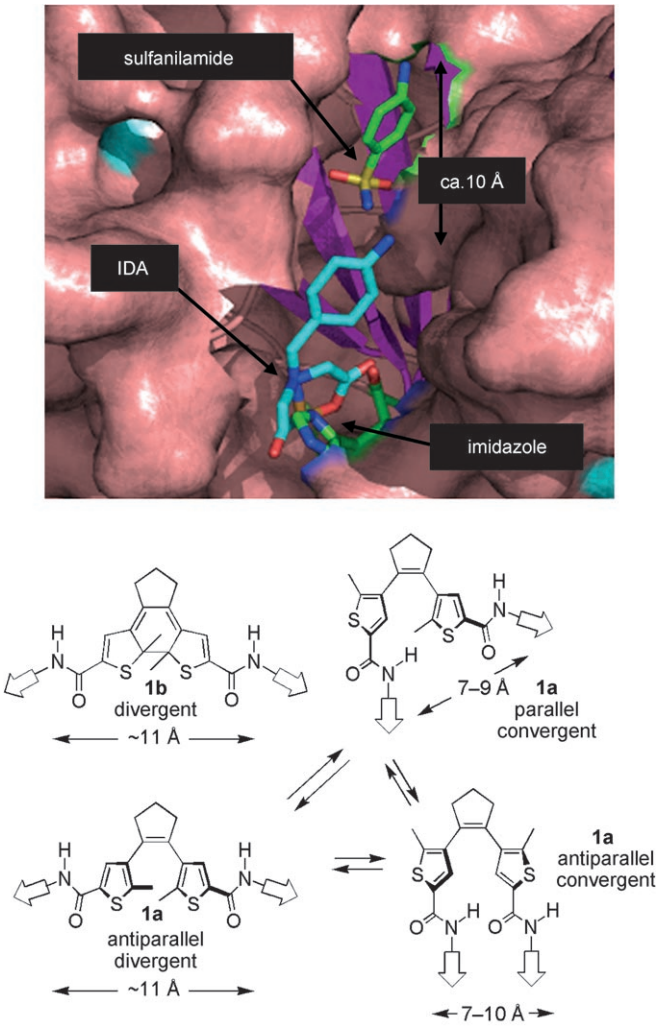


Figure 3. Illustration of the catalytic center of hCAI, containing a sulfanilamide, an IDA, and one of the surface-exposed imidazole groups. The distance between and relative positioning of the sulfanilamide and IDA groups can only be satisfied by the ring-open form of compound **1**, which can adopt the productive parallel conformation. The structure of the enzyme with sulfanilamide in the active site was derived from crystal structure data, generated and rendered with the program PYMOL from Graph Pad.[23] From Mallik's results[14] the length of the inhibitor to guarantee a high binding affinity is known.

We have demonstrated that by using a well-designed, two-pronged inhibitor and appropriate wavelengths of light, the enzyme activity can be reversibly and significantly enhanced by toggling the DTE between a high- and a low-affinity conformation. The thermal stability, nearly quantitative formation of each photoisomer, and activation with visible light makes the system a suitable tool for the reversible

regulation of enzyme activity by light. The use of visible light to activate the inhibitor is particularly important as it will allow better penetration into tissue and reduce the amount of damage caused by higher energy UV light.

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