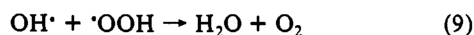
considered. Molecular oxygen scavenges the hydrogen atoms, forming OOH radicals (eq 7) and lowering the recombination of OH$^\bullet$ and H$^\bullet$ radicals (eq 8).

$$H^\bullet + O_2 \rightarrow {}^\bullet OOH \quad (7)$$

$$H^\bullet + OH^\bullet \rightarrow H_2O \quad (8)$$

Consequently more hydroxyl radicals are available if they escape from the bubble (high frequency) in the medium. At low frequency, in the long-lived bubble, OH$^\bullet$ can be scavenged by reaction with the hydroperoxyl radical (eq 9); consequently H_2O_2 and I_3^- formation are more effective under argon.[16]



$$OH^\bullet + {}^\bullet OOH \rightarrow H_2O + O_2 \quad (9)$$

Changing the frequency of ultrasonic waves then produces modifications in the behavior of the species first formed in water sonolysis. This finding constitutes the first example of a clear frequency effect and should have important consequences for optimizing sonochemical oxidation yields.

Registry No. TMP, 2896-70-0; TMPone, 826-36-8; H_2O, 7732-18-5; O_2, 7782-44-7; HO$^\bullet$, 3352-57-6; I^-, 20461-54-5; I_3^-, 14900-04-0; H_2O_2, 7722-84-1.

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Photoswitchable Binding of Substrates to Proteins: Photoregulated Binding of α-D-Mannopyranose to Concanavalin A Modified by a Thiophenefulgide Dye

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Macromolecules exhibiting photoswitchable physical or chemical properties are extensively examined as information storage and signal amplification materials. Photoregulated "on-off" biomaterials provide a novel means to design targeted therapeutic agents activated and deactivated by external light signals. Various means to photoregulate biotransformations by light-switchable enzymes have been described and include the modification of the enzyme active site[1–4] and protein backbone[5,6] by photochromic components and immobilization of enzymes in photochromic copolymers.[7] Here we wish to report on the photoregulation of the binding properties of a protein by its chemical modification with photochromic units. We describe the photoswitchable binding of saccharides to concanavalin A modified by thiophenefulgide dye.

Concanavalin A (Con A) is a globular lectin composed of four subunits (MW = 26K).[8] Each subunit includes binding sites for

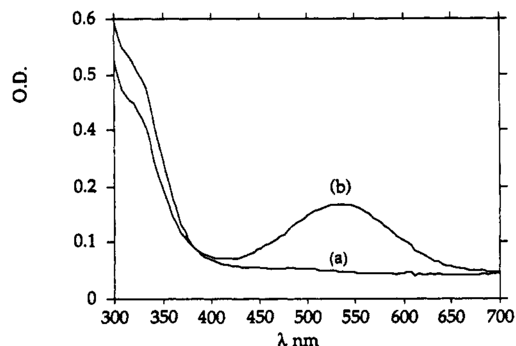

Figure 1. 2-modified Con A: (a) 3-*E* obtained after irradiation of 3-*C* at λ > 475 nm; (b) 3-*C* obtained after irradiation of 3-*E* at 400 nm > λ > 300 nm.

Scheme I

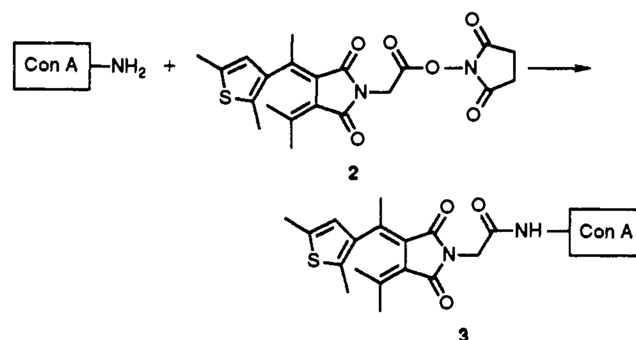

Table I. Association Constants of 4 to 2-Modified Con A in Its Two Photochromic States (*E* and *C*) as a Function of Loading Degree by the Photochromic Material

loading degree^a	dye configuration	$K_a \times 10^{-4}$ (M^{-1})^b
0		2.2
6	*E*	1.64
6	*C*	2
9	*E*	0.78
9	*C*	1.21
12	*E* or *C*	0.64

^{a}Loading degree is defined as the number of photochromic components anchored to each protein backbone. The total number of lysine residues in Con A corresponds to 12, and thus loading degree corresponds to the number of substituted lysine residues. $^b K_a$ = [(Con A)···(4)]/[Con A][4]. Association constants were determined by equilibrating Con A or 3-*E* or 3-*C* (30 μM) with 10 μM 4 in phosphate buffer solution (pH 7) of 0.0875 M, 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$, and 0.1 M NaCl at 20 °C. The protein was filtered off, and the concentration of free 4 was determined by HPLC (RP-18 column, eluent water/methanol, 50:50, at flow rate 1 mL/min, UV detection).

Mn^{2+} and Ca^{2+} and monosaccharide binding sites.[9,10] The metal ions act cooperatively in the association of the monosaccharides to Con A. The affinity of Con A toward monosaccharides is sensitive to the steric configuration of the pyranose hydroxyl groups at positions C-3, C-4, and C-6 of the saccharide.[11] Two pyranoses, α-D-mannopyranose and α-D-glucopyranose, are recognized for binding by Con A, and the affinity of Con A toward α-D-mannopyranose is ca. 3.5-fold higher than toward α-D-glucopyranose.[12] Thus, small steric alterations of the protein backbone

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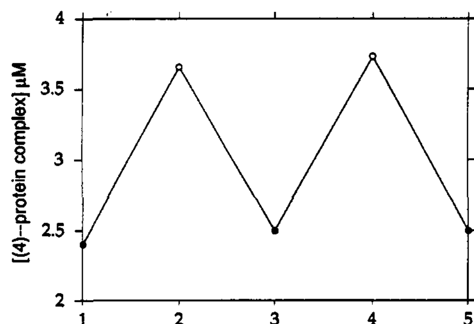
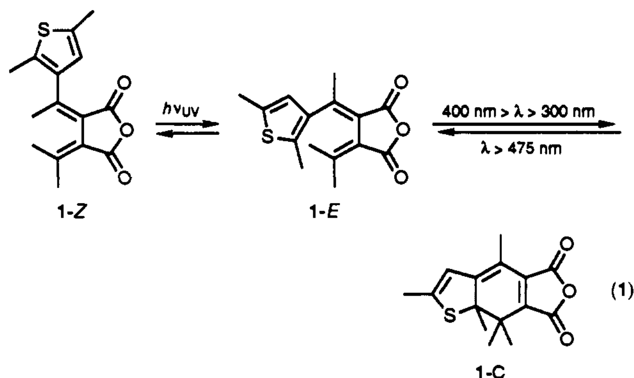


Figure 2. Reversible photoregulated complexation of **2**-modified Con A to **4**. Points 2 and 4 (O) correspond to complexation states of the substrate when **2**-modified Con A is in the *C* form. Points 1, 3, and 5 (●) correspond to decomplexation states when **2**-modified Con A is in the *E* form.

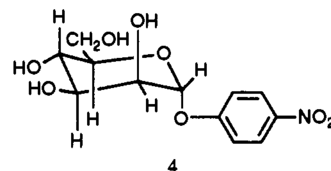
are anticipated to affect its binding properties toward these pyranoses.

Fulgide dyes are well established photochromic materials.[13] Thiophenefulgide (**1**)[14] undergoes three photostimulated isomerization processes[15] (eq 1) where UV irradiation of **1**-*Z* results in **1**-*E* that undergoes subsequent conrotatory electrocyclization to **1**-*C*. Reversible isomerization of **1**-*C* to **1**-*E* is photoinduced



by filtered light, $\lambda > 475$ nm.[16] Con A (Sigma) was modified by thiophenefulgimide (**2**).[17] The modification process was carried out by reacting 50 mg of Con A in 6 mL of an aqueous solution that contained 250 mg $NaHCO_3$ with 5-15 mg of **2** dissolved in 200 µL of THF at 0 °C for 24-48 h (Scheme I). This modification leads to functionalization of Con A lysine residues, and the loading degree is controlled by the amount of added **2** and the reaction time. The resulting modified protein, **3**, exhibits photochromic properties (Figure 1). Illumination of **3**-*E*, 300 nm $< \lambda <$ 400 nm, results in isomerization to **3**-*C*. Further illumination of **3**-*C*, $\lambda > 475$ nm, restores the original absorption spectrum of **3**-*E*. The loading degrees of Con A by **2** are determined by following the absorbance of **3**-*C* at $\lambda = 532$ nm ($\epsilon = 2200$ M^{-1} cm^{-1}) and determining the protein content or by comparing the fluorescence intensities resulting from the interaction of fluorescamine[18] with **3**-*E* and unmodified Con A. Both methods give excellent agreement (±5%).

Photoregulated association of 4-nitrophenyl α-D-mannopyranoside (**4**) to **3**-*E* and **3**-*C* has been followed by determination of the association constants of **4** to the photochromic protein. Table I summarizes the association constants of **4** to Con A in its two photochromic states as a function of loading degree. It is evident that, as the loading degree of Con A increases, its



affinity for **4** declines and the association constant decreases in its value. It is also concluded that, up to a loading degree of 9, the affinity of **3**-*C* is higher than that of **3**-*E* toward binding of **4**. At a loading degree of 12, where all lysine residues of Con A are modified by **2**, a substantial decrease in the binding constant of **4** is observed, and no difference between states *E* and *C* is detected. The largest difference in binding constants of **3**-*E* and **3**-*C* to **4** is observed at a loading degree of 9. This difference in the binding constants allows us to reversibly photoswitch the association and dissociation of **4** to **2**-modified Con A (Figure 2). We see that in the presence of **3**-*E*, only 2.4×10^{-6} M of **4** is bound. Upon illumination, $\lambda = 300$-400 nm, and isomerization to **3**-*C*, enhanced binding of **4** is observed. Further illumination, $\lambda > 475$ nm, regenerates **3**-*E* and the intermolecular complex is dissociated.

We thus conclude that chemical modification of Con A by thiophenefulgimide (**2**) allows the photoinduced binding of 4-nitrophenyl α-D-mannopyranoside to the protein. Photoswitchable association and dissociation of the protein-substrate assembly is induced by the two photochromic states of the protein. Further studies on photoregulation of the binding of substrates to other proteins using this approach are in progress in our laboratory.

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Registry No. Thiophenefulgide, 118408-54-1; α-D-mannopyranose, 7296-15-3.

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Reaction of (6*R*)-6-F-EPSP with Recombinant *Escherichia coli* Chorismate Synthase Generates a Stable Flavin Mononucleotide Semiquinone Radical

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Chorismate synthase (EC 4.6.1.4), the seventh enzyme in the shikimate pathway,[1] catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (**1**, EPSP) to chorismate **2**.[2] The reaction involves the removal of the C-6 *pro-R* hydrogen and loss of

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