Photoregulated Binding of Spiropyran-Modified Concanavalin A to Monosaccharide-Functionalized Self-Assembled Monolayers on Gold Electrodes

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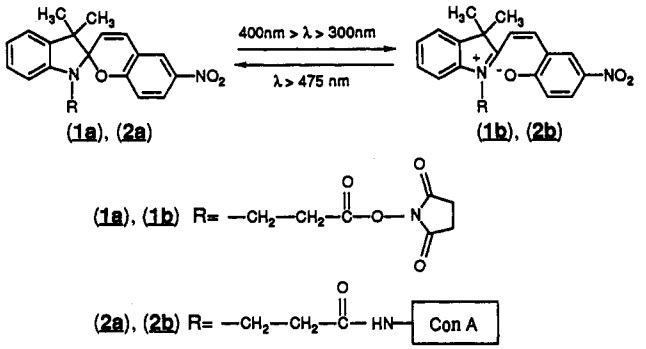
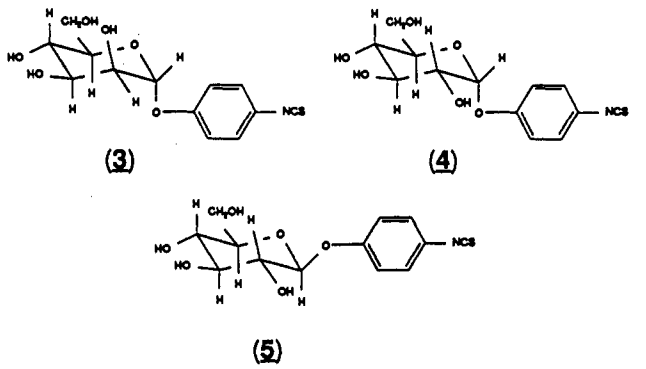
Light-regulated molecular assemblies gain increasing interest within the general subject of molecular devices.[1-3] Light-controlled "On–Off" protein systems provide a fundamental venue for bioelectronic materials.[4,5] Various applications of such systems as targeted therapeutic agents, light signal amplifiers, reversible biosensors, and information processing devices are envisaged.[5] Different methodologies to photoswitch the activities of biomaterials have been developed, including chemical modification of proteins (i.e., enzymes) by photoisomerizable components,[5,6] application of photochromic substrates,[7] and immobilization of proteins in photoregulated polymers.[8] Recently we have reported on the photoregulated binding properties of Concanavalin A (Con A) by its chemical modification with photoisomerizable thiophene fulgide units.[9] In all these studies the binding of the substrates to the biomaterial active sites is photoregulated, and for enzymes the photocontrolled substrate association yields photoswitchable catalytic activities. Here we wish to report for the first time that also the kinetics of substrate association to the biomaterial can be photocontrolled. In this report we follow the photocontrolled kinetics of association of a spiropyran-modified Con A to monosaccharide monolayers organized on gold electrodes. The electrode-associated monolayers of monosaccharides provide an electrochemical means to follow the photoregulated binding processes of the protein.[10]

Concanavalin A (Sigma) has been modified by substitution of its lysine residues with the *N*-hydroxysuccinimide ester of *N*-propionic acid spiropyran, **1a**.[11] The resulting spiropyran-modified Concanavalin, **2a**, exhibits reversible photochromic properties.[12] Illumination of **2a**, 400 nm > λ > 300 nm, results in the formation of the zwitterionic merocyanine-modified Con A, **2b**. Illumination of **2b**, λ > 475 nm, restores **2a**. The average loading degree of **2a** by spiropyran units has been determined to be 6 dye units per protein molecule, using the trinitrobenzene sulfonic acid (TNBS) method.[13]



Monosaccharide monolayers, associated with Au electrodes, have been prepared by the sequence of processes outlined in Scheme I:[14] polished Au electrodes (area corresponding to 3 × 10^{-2} cm^2) were treated with an aqueous solution of cystamine (0.02 M). The resulting self-assembled cystamine monolayer-modified electrodes were then modified by one of the functionalized monosaccharides: *p*-isothiocyanatophenyl α-D-mannopyranoside (**3**), *p*-isothiocyanatophenyl α-D-glucopyranoside (**4**), or *p*-isothiocyanatophenyl β-D-glucopyranoside (**5**) (1 mg of



isothiocyanatophenyl monosaccharide in 300 mL of phosphate buffer, pH = 7.3), to yield the thiourea–monosaccharide monolayer-modified electrodes of phenyl α-D-mannopyranoside (**6**), phenyl α-D-glucopyranoside (**7**), and phenyl β-D-glucopyranoside (**8**). It is known that **6** and **7** bind to specific sites of Con A, while **8** associates less strongly and to a different region of the protein.[15]

The kinetics of **6–8** binding to spiropyran-modified Con A **2a** and the zwitterion **2b** have been followed by cyclic voltammetry.[10a] The modified electrodes of **6–8** have been immersed into a phosphate buffer solution (0.1 M, pH = 8) that contains KCl (1 × 10^{-3} M) as electrolyte and $Fe(CN)_6^{4-}$ (1 × 10^{-3} M) as electroactive group that probes the protein association process. In the absence of the protein, **6–8**-modified electrodes reveal the characteristic reversible redox wave for the ferricyanide/ferrocyanide couple. Upon addition of spiropyran-modified Con A (**2a**) or zwitterion **2b**, a time-dependent decrease in the amperometric response of the cyclic voltammogram is observed. This amperometric decrease is due to binding of the protein to the substrate monolayer associated with the Au electrodes and electrode insulation towards the solubilized redox probe.[16] Thus, the rate of decrease in the amperometric response of the substrate-modified electrode reflects the kinetics of protein association.

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(16) Immobilization of proteins, i.e., bovine serum albumins, to the cystamine-modified Au electrode insulates the electrode toward electrical interaction with the redox probe that is solubilized in the solution.

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Scheme I

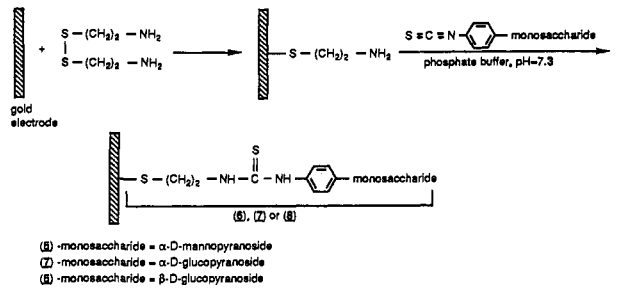

Figure 1 exemplifies the decrease of cathodic currents of the redox probe as a function of time, observed upon interacting **2a** and **2b** with thiourea-derivatized phenyl α-D-glucopyranoside monolayer (**7**). For comparison, the cathodic current decrease in the presence of native Con A is also given. Table I summarizes the time constants ($\tau_{1/2}$) for association of **2a** and **2b** to the various substrates, as deduced from similar electrochemical experiments. For comparison, the kinetic data for association of native Con A to the substrates are also provided. Several conclusions are evident from these results. The kinetics of association of spiropyran-modified Con A to **6**- and **7**-modified monolayers is photoregulated. For example, $\tau_{1/2}$ for binding of **2b** to **6**–monolayer is ca. 2.5-fold longer than that of the neutral spiropyran protein form, **2a**, to **6**–monolayer.[17] Also, it is evident that for **6**– and **7**–monolayers the kinetics of association of the dye-modified protein **2a** or **2b** is slower than that of the native protein to these substrates. In contrast, the results with **8**-modified monolayer reveal that the rates of association of **2a** and **2b** are almost identical and are similar to the rate of binding of native Con A to this substrate monolayer. These results are attributed to the photocontrolled binding of the substrates to the spiropyran-modified Con A: substrates **6** and **7** associate to specific binding sites of the protein.[15] Photoinduced isomerization of **2a** to **2b** results in a steric distortion of the protein backbone[18,19] by means of steric and electrostatic interactions, and, consequently, the association rates are affected. For substrate **8**, which does not exhibit specific binding sites to Con A, the association rates are not influenced.

(17) Photoregulated binding of **6** to spiropyran-modified Con A is observed. While **2a** represents the "On" position for binding, **2b** is the "Off" position of the association process.

(18) Isomerization of **2a** to **2b** is accompanied by a shrinkage of the protein backbone. Time-resolved light scattering experiments reveal that this dynamic conformational shrinkage process proceeds within 150 μs for Con A loaded by six spiropyran units. Willner, I.; Zahavy, E.; Rubin, S., to be published.

(19) The association constants of **2a** and **2b** to *p*-nitrophenyl α-D-mannopyranoside were examined by the method described elsewhere.[9] K_{assoc} of **2a** to Con A is 1.5-fold higher than that of **2b**. $K_{assoc} = 1.8 \times 10^4$ M^{-1} for **2a**, $K_{assoc} = 1.2 \times 10^4$ M^{-1} for **2b**.

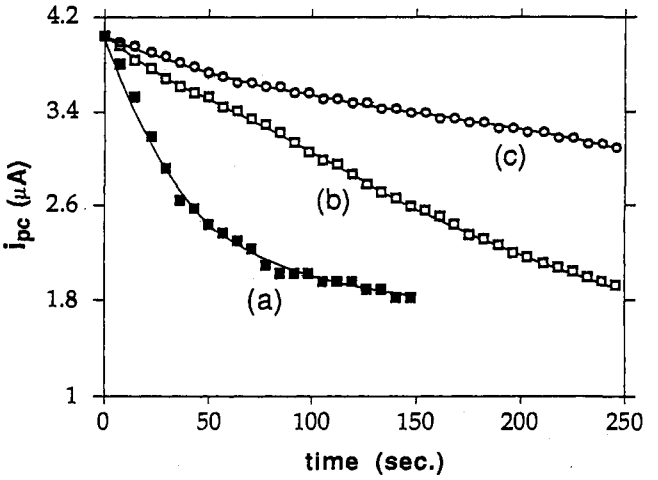

Figure 1. Decay of cathodic current, i_{pc}, of $Fe(CN)_6^{4-}$ redox probe upon interaction of 7–monolayer Au electrode with: (a) native Con A, (b) **2a**, and (c) **2b**. All experiments were performed in a three-electrode cell using Ag/AgCl as reference electrode. Electrolyte composition: 1×10^{-3} M $K_4Fe(CN)_6$, 1×10^{-3} M KCl in phosphate buffer, 0.1 M (pH = 8). Concentration of added protein is 0.01 mg mL^{-1}. All experiments were performed at 20 ± 1 °C, scan rate 200 mV s^{-1}.

Table I. Time Constants $\tau_{1/2}$ (s) for Association of Con A and Spiropyran-Modified Con A to Various Substrates[a]

substrate	$\tau_{1/2}$(Con A)	$\tau_{1/2}$(**2a**)	$\tau_{1/2}$(**2b**)
6	40	60	160
7	85	220	670
8	100	100	110

[a] $\tau_{1/2}$ represents the decay time of cathodic peak current, i_{pc}, to half of its original value in the cyclic voltammogram of the $Fe(CN)_6^{4-}$ redox probe. This time represents the duration to reach half-coverage of the respective monolayer by the protein.

In conclusion, we reveal that photoisomerization of spiropyran units covalently linked to Con A leads to photoregulated binding kinetics of the substrate. We attribute these photostimulated binding processes to the perturbation of the protein binding site in configuration **2b** by electrostatic interactions and steric distortion of the protein backbone.

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Supplementary Material Available: UV–visible spectrum of the photochromic spiropyran modified protein (1 page). Ordering information is given on any current masthead page.