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CONFORMATIONAL DYNAMICS ASSOCIATED WITH PHOTOSWITCHABLE BINDING OF SPIROPYRAN-MODIFIED CONCANAVALIN A

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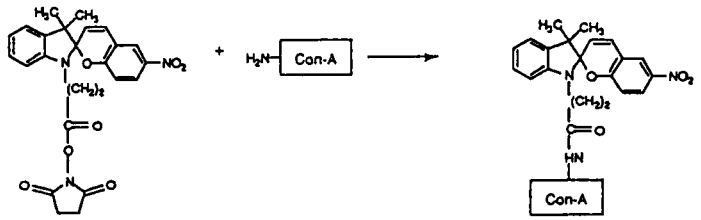
Abstract Spiropyran modified concanavalin A, Con. A, reveals photoregulated binding towards monosaccharides. The association constants of the substrate towards the spiro-Con. A and the kinetics of the association processes are photostimulated. These photostimulated properties are originated from a structural perturbation of the protein upon photoisomerization of spiro-Con. A to the zwitterionic Con. A. Time resolved light scattering reveals that the structural change of the protein involves a shrinkage.

Photoregulation of the functionalities of proteins is of basic interest in future developments of bioelectronic devices[1,2]. One important application involves the development of reversible biosensors, where switchable binding of the analyte to the biological receptor material could lead to reversible use of the sensor. Reversibsle photostimulation of the binding properties of proteins could be designed by chemical modification of the receptor protein by photoisomerizable components[3,4]. Here the protein retains its tertiary structure in the isomer state A, but its structure is distorted and consequently its binding site is perturbed upon photoisomerization to state B. Illumination of the distorted structure and reisomerization to state A resotres the active binding structure of the protein towards its substrate. Here we describe the photostimulated binding of monosaccharides to the lectin concanavalin A, Con. A, that is modified by spiropyran photoisomerizable units[5] and the control of the kinetics of association of the monosaccharides to the photoactive protein[6]. We also provide insight into the structural changes of the protein and the dynamics associated with the structural perturbation of the protein occurring upon isomerization of the photoisomerizable components[5], using time-resolved light scattering experiments.

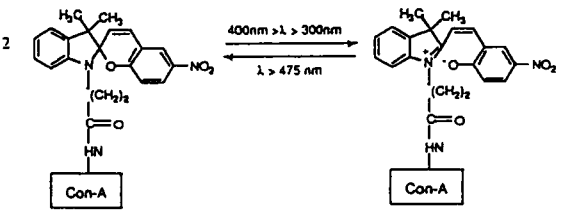
Con. A was modified by the N-hydroxysuccinimide ester of N-propionic acid spiropyran, (1), (pH=8.5, 4°C, 20-30 h) to yield the spiropyran modified Con. A, eq. 1. The extent of loading of the protein by spiropyran units could be controlled by the amount of active ester (1) that was employed in the chemical modification. The spiropyran modified Con. A exhibits reversible photoisomerizable properties. Illumination of (2a), 300 nm$<\lambda<$400 nm results in the formation of (2b), and irradiation of (2b), $\lambda>475$ nm, restores (2a), eq.

Eq. 1

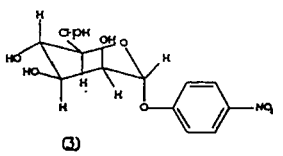


(1)

Eq. 2



(2a) (2b)



(3)

2. The binding constants of p-nitrophenyl-α-D-mannopyranoside, (3), to (2a) and (2b) at different loadings of spiropyran units are summarized in Table 1, and compared to the association constant of (3) to native Con. A. The binding constants of (3) to the spiropyran modified Con. A are lower than to the native protein. The highest difference in association of (3) to (2a) and (2b) is observed with a loading corresponding to 6. We realize that the spiropyran modified Con. A (2a), exhibits a higher affinity towards (3) as compared to the zwitterioonic-merocyanine modified protein (2b). These different association properties of (3) to (2a) and (2b) are attributed to the influence of the photoisomerizable components on the binding sites of the protein[7]. The zwitterionic components in (2b) perturb the protein binding sites and consequently reduce their affinities for (3).

A direct insight into the configurational changes of the protein occurring upon photoisomerization of (2a) to (2b) is obtained by transient light-scattering experiments.

TABLE I: Association constant of spiropyran modified Con. A, in the two photoisomerizable states (2a) and (2b), and time constants for protein conformational relaxation upon photoisomerization of (2a) to (2b).

Loading degree	$K_a(2a)M^{-1}$	K_a (2b) M^{-1}	$\tau_{1/2}$, μs
0	24000		-
3	23000	23000	-
6	18000	12000	$\tau_1 = 60\mu s$
8	10000	7300	$\tau_1 = 60\mu s$, $\tau_2 = 250\mu s$

The scattered light intensity from the protein is expressed by the Debye theory[8], eq. 3 and eq. 4, where R_θ is the scattered light intensity at the angle θ, M_w and C correspond to the molecular weight and concentration of the protein, respectively, $\langle R_G^2 \rangle$ is the mean square of radius gyration of the protein, λ is the wavelength of incident light and A is the second virial coefficient. The constant K is given by eq. 4, where n is the index of refraction of the medium and N is Avogadro's number. Hence, a protein shrinkage (decrease

$$\text{(eq. 3)} \quad \frac{K \cdot C}{R_\theta} = \frac{1}{M_w} \left(1 + \frac{16\pi^2}{3\lambda^2} \langle R_G^2 \rangle \sin^2 \theta/2\right) + A$$

$$\text{(eq. 4)} \quad K = 2\pi^2 n^2 \frac{dn^2}{dc} / N \cdot \lambda^4$$

in the radius of gyration) results in an increase in the scattered light intensity. Photoisomerization of (2a) to (2b) proceeds on a time-scale of 20 ns[9]. Thus any configurational shrinkage (or expansion) of the protein following the photoinduced isomerization is reflected by a transient increase (or decrease) of the scattered light intensity. Figure 1 shows the transient changes in scattered light intensity upon photoisomerization of (2a) to (2b) at loading corresponding to 6 (trace (a)) and 8 (trace (b)). The increase in scattered light intensity implies that upon isomerization of (2a) to (2b) the protein undergoes shrinkage. At a loading degree of 6 the shrinkage proceeds with a time-constant of τ = 60μs. At a loading of 8 the transient increase in scattered light fits two exponentials and reveals a biphasic dynamic shrinkage. One time constant (τ_1 = 60 μs) proceeds to a compact configuration, presumably similar to that observed with the protein of loading of 6. This metastable configuration undergoes further shrinkage (as evidenced by the higher scattered light signal) with a time-constant of τ_2 = 250 μs.

The kinetics of association of (2a) and (2b) to monosaccharides is also controlled by the photoisomerizable cmponents[6]. A series of monosaccharide functionalized self-assembled

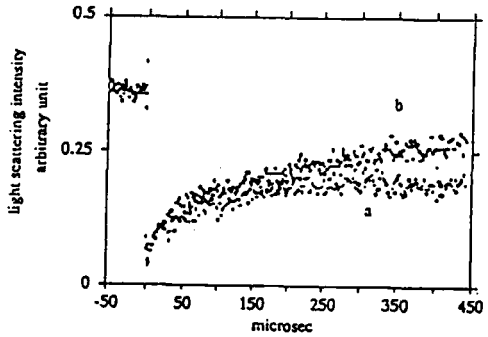


Figure 1: Transient light scattering traces obtained upon photoisomerization of (2a) to (2b) 1mg protein per 1ml phosphate buffer 0.1 M , pH=7.0 , that contains $MnCl_2$, $CaCl_2$ at $1*e^{-4}$ M and NaCl ,0.1M . (a) Loading of 6 . (b) Loading of 8 .

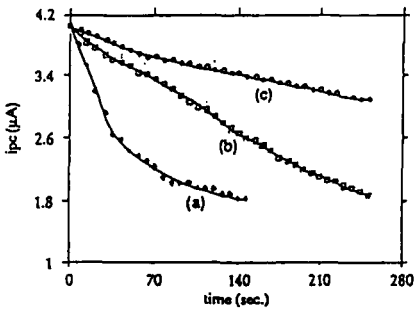
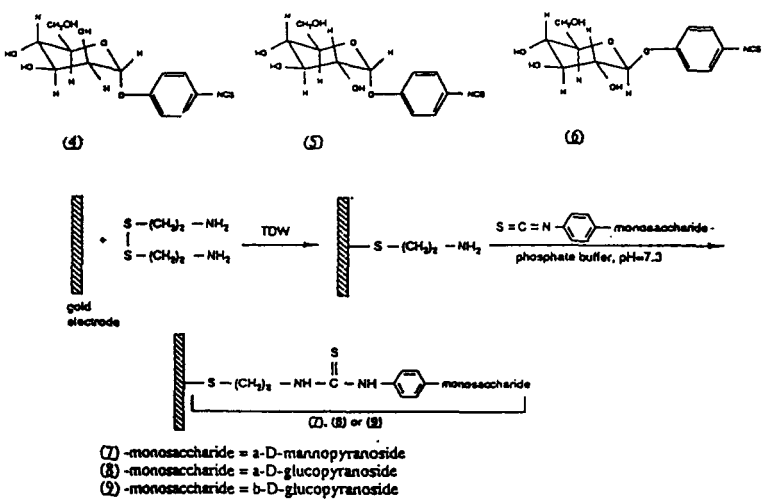


Figure 2: Decay of cathodic current, ipc, of $K_4Fe(CN)_6$ redox probe upon interaction (8) - monolayer Au-electrode with: (a) native Con A (b) with (2a) (c) with (2b). All experiments were performed in a three electrode cell using Ag/AgCl as reference electrode. Electrolyte composition 1x10-3 M $K_4Fe(CN)_6$. 1x10-3 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 °C, scan rate 200 mV s^{-1}.

monolayer, (SAM), electrodes was prepared according to Scheme 1. Au-electrodes were modified by a SAM monolayer of cystamine. The monosaccharides, p-isothiocyanatophenyl-α-D-mannopyranoside, (4), p-isothiocyanatophenyl-α-D-glucopyranoside, (5), and p-isothiocyanatophenyl-β-D-glucopyranoside, (6), were then coupled to the functionalized monolayer to yield the thiourea bridged monosaccharide monolayer, (7), (8) and (9), respectively.



Scheme 1.

The kinetics of association of the two photoisomerizable proteins, (2a) and (2b), to the monosaccharide monolayer was probed electrochemically. For this purpose the redox couple $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ was introduced to an electrochemical cell that includes the different monolayer modified electrodes as a working electrode (electrolyte solution, KCl, $1x10^{-3}$ M,

in phosphate buffer, 0.1 M, pH=8). Injection of the proteins (2a) or (2b) to the electrochemical cell results in their association to the monosaccharide monolayer. Binding of the protein insulate the electrode towards the redox probe in solution, and thus the decrease in the amperometric response of the electrodes as a function of time reflects the association rates of (2a) or (2b) to the respective SAM electrodes of the various monosaccharides. Figure 2 shows the decrease of the cathodic current i_{pc} (of $Fe(CN)_6^{3-}$) upon interacting the SAM electrode that includes (7) with (2a) , (2b) and native Con. A. Table 2 summarizes the time constants for association of the proteins to the various

TABLE II: Time constants for the association of Con. A and spiropyran modified Con.A to different substrates.

Substrate	$\tau_{1/2}$ (Con. A)	$\tau_{1/2}$'s (2a)	$\tau_{1/2}$'s (2b)
7	40	60	160
8	85	220	670
9	100	100	110

electrodes. We realize that binding of (7) and (8) is enhanced to (2a) as compared to the distorted protein (2b). The monosaccharide (9) that does not exhibit specific binding sites to Con. A is not influenced by the photoisomerizable units and (2a), (2b) and native Con. A reveal similar binding kinetics. We thus conclude that upon photoisomerization of (2a) to (2b) the protein backbone undergoes shrinkage. As a result, the binding sites towards monosaccharides are distorted in (2b), as reflected by the lower association constants and decrease in binding rates.

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