Examination of the Conformational Dynamics of Photoregulated Concanavalin A by Time-resolved Light Scattering

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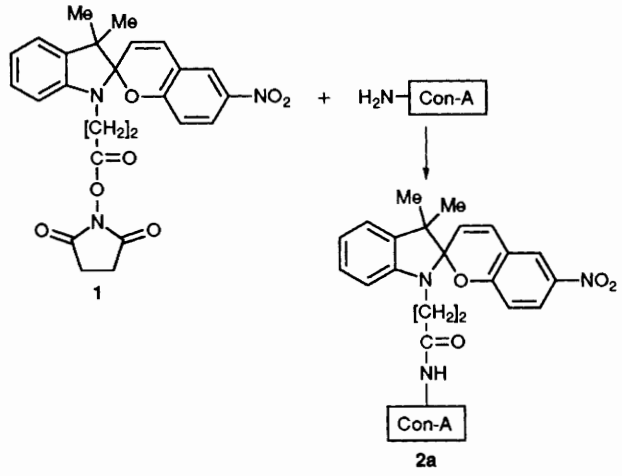
Spiropyran-modified Concanavalin A (Con-A) displays photoregulated binding towards α-D-mannopyranoside, the photostimulated binding of the substrate originating from a structural perturbation of the protein upon photoisomerization of spiro-Con-A to the zwitterionic Con-A; time-resolved light scattering reveals that the structural change of the protein involves a shrinkage, and the dynamics of the protein shrinkage depend on the loading of Con-A with spiropyran units.

Photoregulation of proteins is a basic principle for the development of bioelectronic devices.[1,2] Photoswitchable functions of biomaterials such as enzyme activities[3,4] or protein binding properties[5] could be used in the amplification of weak light signals, the development of reversible biosensors, the design of information storage devices and for the development of site-specific targeted therapeutic materials.[2] Chemical modification of proteins by photoisomerizable components has been suggested as a method for reversible photostimulation of protein functionalities to 'ON–OFF positions.[4,5] For example, azobenzene-derivatized papain displays photoswitchable hydrolytic activities and Concanavalin A, modified by thiophenefulgide photoisomerizable units, displays light-stimulated binding of α-D-mannopyranoside. The origin of the photoswitchable properties of these proteins has been attributed to conformational perturbations of the protein backbones, as a result of the isomerization processes of the covalently linked photoactive units. That is, in one photoisomer state the active site structure of the protein is preserved and the protein is activated towards its biological function (position 'ON'), while in the complementary photoisomer state, the protein structure is perturbed and it is deactivated (position 'OFF'). Nonetheless, in all of these studies the relation between the photoswitchable activities of the proteins and their structural properties is merely speculative. Thus, it is of great interest to provide experimental evidence for such light-induced structural changes of proteins, and specifically to identify shrinkage or expansion processes of proteins as well as to reveal the dynamics associated with such structural changes following photochemical isomerization. Here we report on the photoswitchable binding activities of Concanavalin A modified by spiropyran photoisomerizable components, and highlight the dynamics associated with the structural perturbation of the protein by the photoisomer units.

Concanavalin A (Con-A) was treated with the *N*-hydroxysuccinimide ester of *N*-propionic acid spiropyran **1**,[6] (pH 8.5, 4 °C, 20–30 h), to yield the spiropyran-modified Con-A, **2a**

(Scheme 1). The number of lysine residues of the protein that are modified by spiropyran units is controlled by the amount of spiropyran **1** used in the chemical modification and by the reaction time. Different spiropyran-derivatized Con-A proteins have been prepared with average loadings corresponding to 3, 6 and 8 (out of 12 lysine residues in the protein backbone).[7] Loading degrees were derived using the TNBS method.[8] The spiropyran-modified Con-A exhibits reversible photochromic properties as shown in Fig. 1. The isomer **2a** undergoes photoisomerization ($300 < \lambda < 400$ nm) to the zwitterionic isomer **2b**, $\lambda_{max} = 530$ nm (Scheme 2). In turn, the zwitterionic form **2b**, undergoes photoinduced electrocyclization to **2a** upon illumination with visible light, $\lambda > 475$ nm. The binding constants of *p*-nitrophenyl-α-D-mannopyranoside, **3** to **2a** and **2b** at different loadings of the protein by the photoisomers are summarized in Table 1 and compared with the association constant of **3** to the native protein, Con-A. It is evident that the binding constants of the substrate to the chemically-modified proteins exhibit lower values compared with native Con-A. As the loading of the photoisomerizable component increases, the association constants towards the substrate decrease. Most important, however, is the observation that the two isomer states of the modified protein, **2a** and **2b**, reveal different association constants towards **3**. For example, Con-A with an average loading of 6 has an association constant corresponding to 12 000 mol^{-1} dm^3 in its zwitterionic state and 18 000 mol^{-1} dm^3 in its spiro form. Thus, the spiropyran-derivatized Con-A shows photoswitchable binding properties towards **3**.

An insight into the dynamic events associated with the structural changes of the protein upon photoisomerization of **2a** to **2b** is obtained by transient light scattering experiments. The scattered light intensity from the protein is expressed by the Debye theory,[9] (eqns 1 and 2) where R_θ is the scattered light intensity at the angle θ, M_w and C correspond to the



Scheme 1

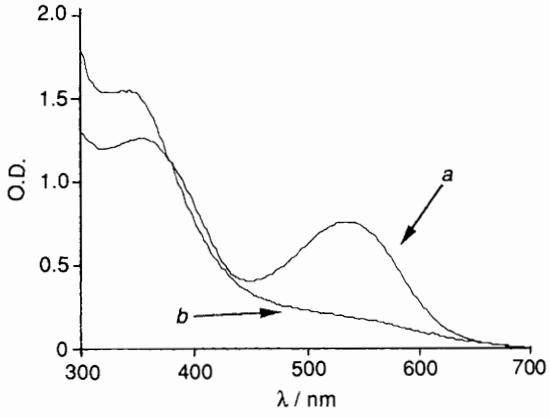
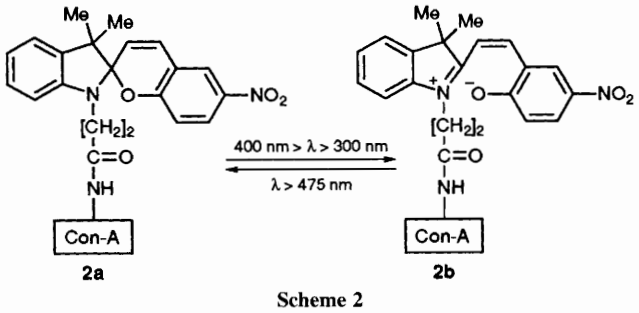


Fig. 1 Absorption spectra of spiropyran-modified Con-A: (*a*) Spectrum of **2b** obtained by illumination of **2a**, 300 nm < λ < 400 nm; (*b*) Spectrum of **2a** obtained by illumination of **2b**, λ > 475 nm



Scheme 2

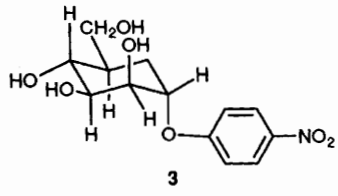


Table 1 Association constants[a] of spiropyran-modified Con-A, in two photoisomerizable states, **2a** and **2b**, and time constants[b] for protein conformational relaxation upon photoisomerization of **2a** to **2b**

Degree[c] of loading	K_a **2a**/mol^{-1} dm^3 (±5%)	K_a **2b**/mol^{-1} dm^3 (±5%)	$\tau_{\frac{1}{2}}$/μs (±3%)
0[d]	24 000	—	—
3	23 000	23 000	—
6	18 000	12 000	$\tau_{\frac{1}{2}} = 60$
8	10 000	7 300	$(\tau_{\frac{1}{2}})^1 = 60$
			$(\tau_{\frac{1}{2}})^2 = 250$

[a] Association constants were derived by following the free substrate by HPLC. [b] All light scattering-transients fit single or two exponential decays. [c] Loading degrees were derived[8] from modification of native and spiropyran-modified Con-A with trinitrobenzenesulfonic acid and following the absorbance at λ 335 nm. [d] Association constant of native Con-A.

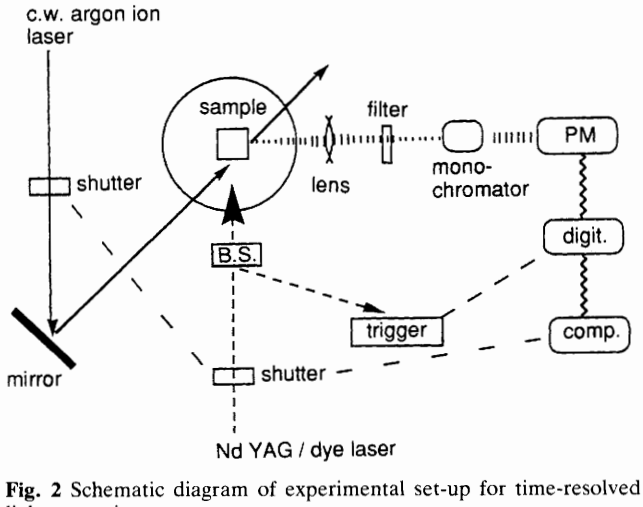


Fig. 2 Schematic diagram of experimental set-up for time-resolved light scattering

molecular weight and concentration of the protein, respectively, $<R_G^2>$ is the mean square of the radius of gyration of the macromolecule, λ is the wavelength of the incident light and A is the second viral coefficient. The constant K is given by Scheme 2, where n is the index of refraction of the medium and N is Avogadro's number.

$$\frac{KC}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{16\pi^2}{3\lambda^2}<R_G^2>\sin^2\theta/2\right) + A \quad (1)$$

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

Hence, a shrinkage of the protein (decrease in the radius of gyration) as a result of the isomerization of the photoactive groups, is associated with an increase in the scattered light intensity as compared with the original state. In turn, an expansion of the protein backbone following photoisomerization is expected to result in a decrease in the scattered light intensity. The photoisomerization of the spiropyran units of **2a** to the zwitterionic isomer **2b** proceeds on a time-scale of 20 nsec.[10] Thus, any conformational change of the protein backbone that follows the fast isomerization process will result in a time-dependent scattered light intensity signal. The time profile of the scattered light intensity will then reflect the dynamic processes associated with structural changes of the protein. Accordingly, the experimental set-up shown in Fig. 2 was designed. It consists of a pulsed Nd–Yag laser coupled to a dye laser that initiates the photoisomerization of **2a** to **2b** (pulse duration *ca.* 15 nsec). Following the isomerization process, the pulsed laser is blocked by a shutter and the sample is exposed to the light of a CW–argon ion laser. The latter CW laser is triggered on with an appropriate delay with respect to the pulsed laser. The scattered light is collected, passed through a monochromator (λ = 455 nm) and detected by the photomultiplier. The scattered light signals are then collected by a digitizer and analysed by a computer.[11] Fig. 3 shows the transients of scattered light intensities that are observed upon light-induced isomerization of **2a** to **2b** at spiropyran loadings corresponding to 6 [Fig. 3(*a*)] and 8 [Fig. 3(*b*)].† No scattered light transients were detected for the native protein or for the protein with the low loading of 3. Clearly, photoisomerization of **2a** to **2b** involves a time-dependent increase in the scattered light that levels off. The final intensity of scattered light of **2b** depends on the loading of the protein with spiropyran units, and a higher loading results in a higher value of scattered light intensity. The increase in scattered light upon isomerization of **2a** to **2b** implies that the protein undergoes shrinkage upon isomerization. Furthermore, the time-dependent increase in scattered light represents the dynamics of the structural changes of the protein backbone upon isomerization of **2a** to **2b**. For **2a** with a loading of 6 the increase in scattered light follows single exponential kinetics, $\tau_{\frac{1}{2}}$ = 60 μsec. In turn, with a loading corresponding to 8 the increase in scattered light intensity fits a biexponential process with time constants of $(\tau_{\frac{1}{2}})^1$ = 60 μsec, and $(\tau_{\frac{1}{2}})^2$ = 250 μsec, and the final protein state exhibits enhanced scattered light intensity as compared with the protein with a loading of 6. These results suggest that isomerization of **2a** to **2b** is always accompanied by a shrinkage of the protein backbone. For the protein of loading 6, the shrinkage upon isomerization to the newly organized protein structure, proceeds with a time constant of τ = 60 μsec. At the higher loading of 8 the final protein structure is in a more shrinked configuration than the former protein. The dynamics of formation of this compact protein structure shows, however, a rapid shrinkage to a metastable protein configuration similar to that observed at the lower loading (τ = 60 μs) followed by a slow (τ = 250 μs) conformational change corresponding to formation of a more compact structure.‡

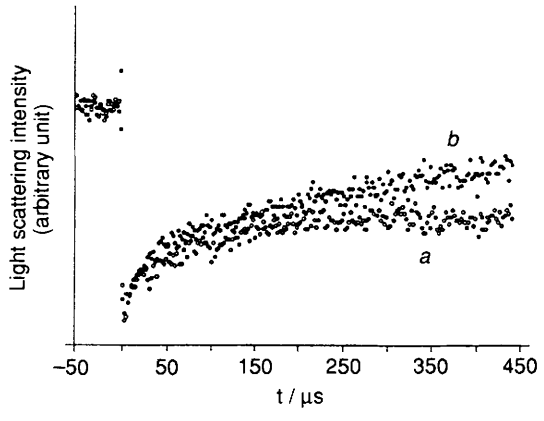


Fig. 3 Transient light scattering traces obtained upon photoisomerization of **2a** to **2b**: 1 mg protein/1 cm^3 phosphate buffer, pH 7.0, that contains $MnCl_2$ 1 × 10^{-4} mol dm^{-3}, $CaCl_2$ 1 × 10^{-4} mol dm^{-3}, NaCl 0.1 mol dm^{-3}: (*a*) Loading of 6; (*b*) Loading of 8

† The fast initial decrease in the scattered light transient is due to absorption of the incident light by **2b** formed upon isomerization.

In conclusion, we reveal the relationship between structural changes of proteins and their photoregulated biological functions. Time-resolved light scattering is suggested as a general method for identification of dynamic structural processes in photostimulated biomaterials.

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‡ The time-resolved light scattering experiments are supported by steady-state light scattering experiments of **2a** and **2b** separately. The difference in scattered light agrees with the initial and final intensities of the time-resolved experiments.