Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Photoregulation of the Activities of Proteins

Shai Rubin [a] & Itamar Willner [a]

[a] Institute of Chemistry and Farkas Center for Light Induced Processes, The Hebrew University of Jerusalem, 91904, Israel

Version of record first published: 24 Sep 2006.

To cite this article: Shai Rubin & Itamar Willner (1994): Photoregulation of the Activities of Proteins, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 246:1, 201-205

To link to this article: http://dx.doi.org/10.1080/10587259408037814

Mol. Cryst. Liq. Cryst. 1994, Vol. 246, pp. 201–205
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United States of America

PHOTOREGULATION OF THE ACTIVITIES OF PROTEINS

SHAI RUBIN and ITAMAR WILLNER

Institute of Chemistry and Farkas Center for Light Induced Processes,

The Hebrew University of Jerusalem 91904, Israel.

Abstract Azobenzene modified papain, reveals photoregulation toward hydrolysis of Nα-benzoyl-DL-arginine-4-nitroanilide. The association constants of thiophenefulgide modified concanavolin A (Con. A) towards monosaccharides is reversibly photoregulated. Immobilized α-chymotrypsin in photochromic copolymers exhibits "ON-OFF" photoregulation towards the hydrolysis of N-(3-carboxpropionyl)-L-phenylalanine p-nitroanilide.

Photostimulation of the activities of proteins to "ON" and "OFF" states is important for the development of targeted therapeutic materials and bioelectronic devices such as reversible biosensors or signal amplification and recording units[1]. We have developed a general methodology to photostimulate proteins by the attachment of reversibly photoisomerizable compounds to the protein backbone, Figure 1. Here, the protein that includes the covalently attached photoisomer state A preserves its active site structure, and hence exhibits its native activity (switch "ON"). Upon photoisomerization to state B, the protein is distorted and its active site is perturbed. This leads to deactivation of the protein (switch "OFF"). Further reversible photoisomerization of state B to state A restores the tertiary structure of the proteins and its native functionality is recovered. This methodology has been successfully applied to photoregulate the hydrolytic activity of the enzyme papain[2] and control the binding properties of concanavalin A towards saccharides[3].

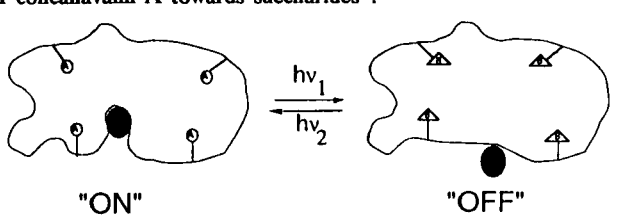
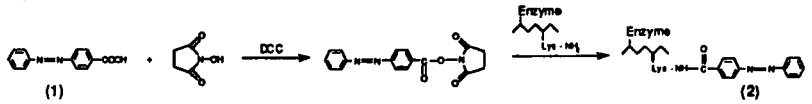


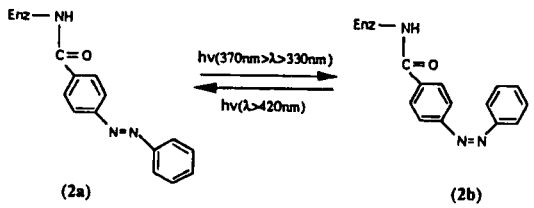
Figure 1. The protein backbone is modified by photochromic groups. The activity of the protein depends on the configuration of the photochromic components.

The enzyme papain was chemically modified by *trans*-4-carboxyazobenzene (1) according to eq. 1. The resulting *trans*-azobenzene modified papain *t*-Az-P, (2a), exhibits reversible photochromic properties and retains 80% of the native enzyme activity. Illumination of *t*-Az-P, 370 nm > λ > 330 nm results in the *cis* isomer, *c*-Az-P, (2b). Further illumination of *c*-Az-P resotres *t*-Az-P (eq. 2). We examined the hydrolytic activities of the azobenzene modified papain in the two

Equation 1

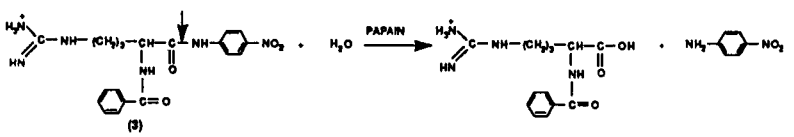


Equation 2



photoisomer states *t*-Az-P and *c*-Az-P (Figure 2(a) and (b)). In this study we employed an enzyme that exhibits a loading of 50%. That is, out of 10 lysine residues comprising the protein, 5 are derivatized by azobenzene units. The rate of hydrolysis of (3) was used to assay the enzyme, eq. 3. Figure 2(a) shows that when the hydrolysis is initiated with the *t*-Az-P, it proceeds

Equation 3



effectively (v=2 $\mu M \cdot min^{-1}$). Upon isomerization of *t*-Az-P to *c*-Az-P (time marked with arrow), the hydrolysis is slowed down (v=0.7 $\mu M \cdot min^{-1}$). Figure 2(b) shows that when the hydrolytic transformation is initiated with *c*-Az-P, a slow reaction takes place. Upon isomerization of *c*-Az-P to *t*-Az-P (time marked with arrow) the hydrolysis is enhanced. The activity of *t*-Az-P towards hydrolysis of (3) is ca. 2.75-fold higher than that of *c*-Az-P. Thus, *t*-Az-P represents the switched-on state of the enzyme, while *c*-Az-P is the switched-off protein.

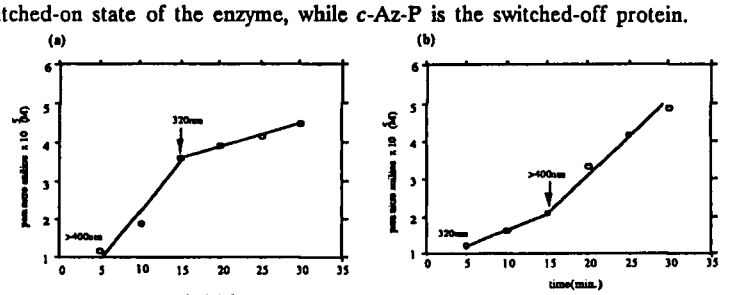
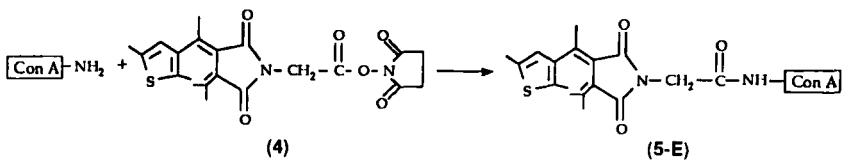


Figure 2. Photoregulated hydrolysis of (3). (a) Hydrolysis is initiated by t-Az-P and switched off by c-Az-P. The arrow indicates photochemical conversion of t-Az-P to c-Az-P. (b) Hydrolysis is initiated by c-Az-P and then accelerated by t-Az-P. The arrow indicates photochemical conversion of c-Az-P to t-Az-P

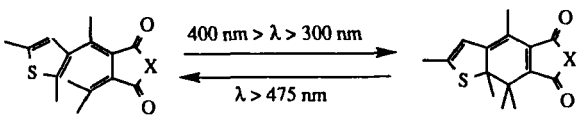
Similarly, the binding properties of concanavalin A, Con. A, towards monosaccharides are photostimulated by covalent linkage to photoisomerizable compounds to the protein. Con. A includes four subunits (MW=26 KD), where each subunit contains a Mn^{2+}, a Ca^{2+} and a monosaccharide binding site. The metal ions act cooperatively in the association of monosaccharides such as α-D-mannopyranose or α-D-glucopyranose.

Con. A was modified with the active ester derivative of thiophenefulgide (4) according to eq. 4. The resulting thiophenefulgide modified Con. A, (5-E), exhibits reversible photochromic properties, eq. 5. Illumination of (5-E) at 400 nm>λ>300 nm results in (5-C), and subsequent

Equation 4



Equation 5



(5-E) (5-C)

X=N-CH_2-CO-NH-Lys-Con A

irradiation of (5-C), λ>475 nm, restores the isomer (5-E). The binding constants of (5-E) and (5-C) towards p-nitrophenyl α-D-mannopyranose (6) depend on the loading of the protein by the photoisomerizable units and the structure of the respective photochromic isomer. For example, at a loading of 9 the binding constant of (6) to (5-E) corresponds to $K_a = 7.8 \times 10^3\ M^{-1}$, while the association constant of (6) to (5-C) is $K_a = 1.2 \times 10^4\ M^{-1}$. This allows us to reversibly switch the binding of (6) to Con. A as shown in Figure 3.

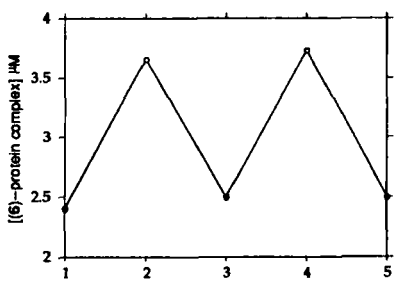


Figure 3. Reversible photoregulated complexation of (4)-modified Con A to (6). Points 2 and 4 (o) corresponds to complexation states of the substrate when (4)-modified Con A is in the C form. Points 1,3, and 5 (•) correspond to decomplexation states when (4)-modified Con A is in the E form.

The photostimulated hydrolytic activites of azobenzene modified papain, and the photoswitchable binding properties oof thiophenefulgide derivatized Con. A originate from steric

perturbations exerted on the protein backbones by the photoisomerizable units. In one of the photoisomer states *t*-Az-P, (2a) and (5-E), the tertiary structure of the respective protein is retained and the biomaterial is in its active form (switch "ON"). In the photoisomer states *c*-Az-P, (2b) and (5-C), the respective proteins are distorted and their active-site is perturbed. This leads to deactivation of the proteins (switch "OFF"). Perturbation of the protein backbones could be induced by dipole-dipole interactions, steric constrains or electrostatic interactions exerted by the photoisomer on the protein skeleton.

An alternative methodology to photostimulate biomaterials involves the immobilization of proteins in the photoregulated environment[4], as schematically exemplified in Figure 4 using a

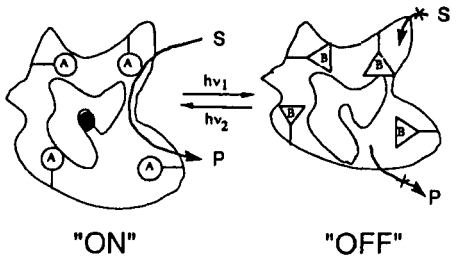
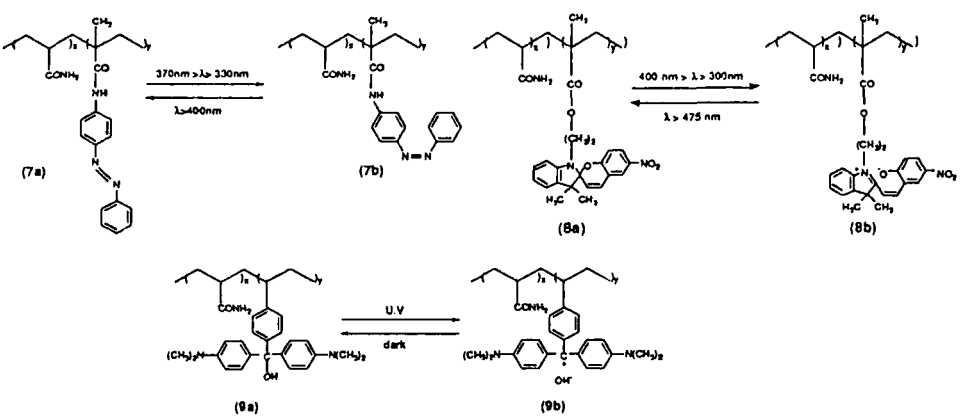


Figure 4. The photochromic polymer is permeable towards the substrate in the ON position. After irradiation of the polymer it become non-permeable towards the substrate (OFF position).

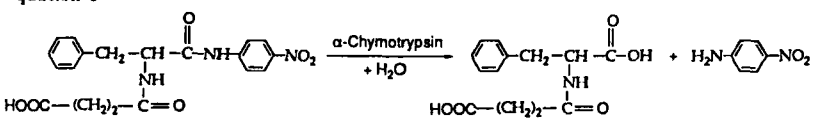
photoisomerizable polymer as entrapment medium for the protein. The wettability[5], viscosity[6], volume and permeability[7] of photochromic polymers are known to be controlled by the isomer state of the photochromic component. Accordingly, entrapment of an enzyme in a substrate non-permeable polymer will result in a non-active biomaterial (switch "OFF"). Upon photoisomerization of the polymer it turns permeable towards the substrate and thus the enzyme is activted for its biological function (switch "ON").

We have applied three different photochromic copolymers (7) - (9), as photostimulated



reaction mediums for the enzyme α-chymotrypsin. The enzyme was immobilized in these photochromic copolymers and its hydrolytic activity was assayed according to eq. 5 in the

Equation 6



respective photoisomer states of the copolymers. Figure 5 exemplifies the photostimulation of α-chymotrypsin immobilized in the spiropyran-acrylamide copolymer, (8). We see that when the

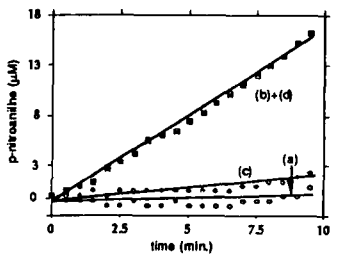


Figure 5. Rate of hydrolysis of (10), by α-chymotrypsin, immobilized in copolymer (8). (a) and (c) Copolymer is in (8a) form. (b) and (d) Copolymer is in (8b) state.

copolymer is in the neutral spiropyran state, (8a), the enzyme is switched off towards hydrolysis of (10). In turn, upon photoisomerization of the copolymer to the zwitterionic state (8b), the enzyme is activated toward hydrolysis of (10). Reversible photoisomerization of the copolymer allows cyclic "ON-OFF" activation and deactivation of α-chymotrypsin. Flow dialysis experiments reveal that the permeability of the substrate, (10), across to the polymer acting as a membrane, is photostimulated by light. The polymer configuration (8a) is non-permeable for the substrate and consequently the enzyme is inactive towards hydrolysis of (10). In the copolymer configuration (8b), the substrate is permeable across to polymer membrane and hence its hydrolysis by α-chymotrypsin proceeds effectively.

Acknowledgement: This research was supported by the Basic Research Foundation administered by the Israel Academy of Sciences and Humanities.

REFERENCES

1. I. Willner, B. Willner, in Bioorganic Photochemistry. Biological Applications of Photochemical Switches, Vol. 2, Wiley, in press.
2. I. Willner, S. Rubin, A. Riklin, J. Am. Chem. Soc., 113, 3321 (1991).
3. (a) I. Willner, S. Rubin, J. Wonner, F. Effenberger, P. Bauerle, J. Am. Chem. Soc., 114, 3150 (1992).
   (b)) I. Willner, S. Rubin, Y. Cohen, J. Am. Chem. Soc., 115, 4937 (1993).
4. (a) I. Willner, S. Rubin, T. Zor, J. Am. Chem. Soc., 113, 4013 (1991).
   (b) I. Willner, S. Rubin, R. Shatzmiller, T. Zor, J. Am. Chem. Soc., in press.
5. M. Irie, R. Iga, Makromol. Chem., Rapid Commun. 8, 569 (1987).
6. M. Irie, K. Hayashi, J. Macromol. Chem., A13, 511 (1979).
7. M. Irie, D. kungwatchakum, Makromol. Chem., Rapid Commun., 5, 829 (1984).