# Reversible Light-Stimulated Activation and Deactivation of α-Chymotrypsin by Its Immobilization in Photoisomerizable Copolymers[1]

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**Abstract:** The enzyme α-chymotrypsin was immobilized in acrylamide copolymers which contain photoisomerizable components. The resulting enzyme–copolymer assemblies reveal photoswitchable "on–off" biocatalytic activities. Three kinds of acrylamide copolymers cross-linked with 4-(methacryloylamino)azobenzene (polymer **1**) 1-[β-(methacryloxy)-ethyl]-3,3-dimethyl-6-nitrospiro[indoline-2,2′-[2*H*-1]benzopyran] (polymer **2**), and bis[4-(dimethylamino)phenyl](4-vinylphenyl)methyl leucohydroxide (polymer **3**) were used to immobilize the enzyme. The enzyme reveals bioactivity (position "on") in the copolymer isomer states **1b**, **2b**, and **3b**, respectively, while its activity is blocked (position "off") in copolymers **1a**, **2a**, and **3a**, respectively. The activity of the enzyme is assayed toward the hydrolysis of *N*-(3-carboxypropionyl)-L-phenylalanine *p*-nitroanilide (**7**). The photostimulated activities of the enzyme entrapped in the different copolymers correlate with the permeability properties of the substrate **7** across the photoisomer forms of the copolymers. While the copolymer isomer forms **1a**, **2a**, and **3a** exhibit poor permeability toward the substrate **7**, the copolymers **1b**, **2b**, and **3b** are permeable toward the substrate **7**.

Photoregulation of protein activities is of general interest for future bioelectronic devices. Amplification of weak light signals, image recording by biocatalytic transformations, reversible biosensor systems, and targeted therapeutic materials are a few potential applications of reversible photoregulated proteins.[2] "On–off" photostimulation of enzyme activities was achieved by covalent attachment of photoisomerizable (photochromic) components to proteins[3-6] and modification of the enzyme active site by photoisomerizable units[7-9] by the use of photoisomerizable inhibitors.[10-12] In a recent preliminary report, we described a novel method by which the activity of α-chymotrypsin can be regulated by its immobilization in a photoisomerizable azobenzene copolymer. The switchable activities of the polymer-immobilized enzyme were attributed to the photostimulated transport of the enzyme–substrate across the polymer matrix in its two photostimulated *cis* and *trans* states.

The photostimulation of physical and chemical properties of photoisomerizable polymers (photochromic polymers) has been examined extensively in recent years.[13] Volume changes,[14] wettability,[15] and viscosity[16] represent a few physical properties that are reversibly controlled in the photoisomerizable polymer assemblies. Also, photoisomerizable polymers were applied as light-stimulated ion carriers in liquid–liquid membrane systems.[17] Accordingly, the encapsulation of proteins in photoisomerizable polymers could provide a general means of photoregulating enzymes by means of light-induced permeability of the substrate across the polymer membrane. This approach could be classified as a photostimulated medium effect on the activities of the biomaterials.

Here we wish to report in detail on the reversible photoregulation of α-chymotrypsin in three different photochromic acrylamide copolymers: 4-(methacryloylamino)azobenzene (**1**), 1-[β-(methacryloxy)ethyl]-3,3-dimethyl-6′-nitrospiro[indoline-2,2′-[2*H*-1]-benzopyran] (**2**), and bis[4-(dimethylamino)phenyl](4-vinylphenyl)methyl leucohydroxide (**3**). We reveal that the photostimulated activities of the polymer-encapsulated enzyme originate from the light-controlled permeabilities of the substrate across the polymer matrices.

## Experimental Section

The photoisomerizable monomers 4-(methacryloylamino)azobenzene[18] (**4**), 1-[(β-(methacryloxy)ethyl]-3,3-dimethyl-6′-nitrospiro[indoline-2,2′-[2*H*-1]benzopyran][19,20] (**5**), and bis-[4-(dimethylamino)phenyl](4-vinylphenyl)methyl leucohydroxide[21] (**6**) were prepared according to the reported procedures. α-Chymotrypsin (E.C. 3.4.21.1) and all other chemicals were obtained from commercial sources.

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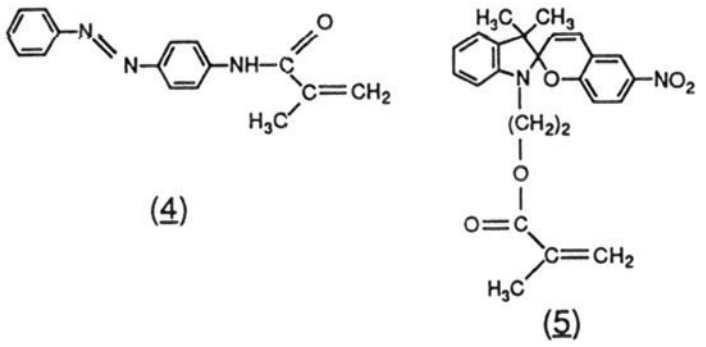

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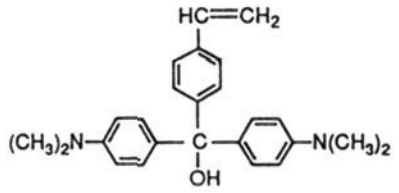

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Immobilization of the enzyme in the acrylamide photochromic copolymers **1–3** was performed as follows. Acrylamide, 375 mg (5.3 mmol), 20 mg (0.13 mmol) of *N,N'*-methylenebis(acrylamide), and 15 mg (585 units) of α-chymotrypsin were dissolved in 1 mL of distilled water. Samples, 0–1 mol %, of the photoisomerizable monomers **4**, **5**, or **6** were dissolved in a minimum quantity (0.5–1 mL) of DMSO and mixed with the enzyme–acrylamide aqueous solution. The resulting mixture was degassed with argon, and 0.5 mL of a 1% (w/w) potassium peroxodisulfate aqueous solution and 0.25 mL of a 50% (v/v) 3-(dimethylamino)propionitrile aqueous solution were injected into the mixture to initiate the polymerization. Polymerization took place within a few minutes, and the polymer mixture was left undisturbed with no further treatment for 1 h. The resulting polymers **1–3** were washed with water and kept in an aqueous solution in the refrigerator overnight.

The photoisomerizable copolymer was cut into slices 2-mm thick, each slice containing 47.6 units of immobilized enzyme.

**Enzymatic Assay.**[22] The immobilized α-chymotrypsin was assayed by measuring the hydrolysis rate of *N*-(3-carboxypropionyl)-L-phenylalanine *p*-nitroanilide (**7**). The polymer was cut into slices 2-mm thick which were placed in a quartz cell containing 0.3 mL of buffer (triethanolamine 0.2 M, pH = 7.8, $[Ca^{2+}]$ 20 mM) and 0.3 mL of substrate solution (5.7 mM of **7** in triethanolamine 0.2 M, pH = 7.8). The copolymer was incubated for 2 min (25 °C), and then formation of nitroaniline ($\lambda$ = 405 nm, $\epsilon$ = 10 200 $M^{-1}\cdot cm^{-1}$) was followed spectroscopically.

The flow-dialysis experiments[23] were performed in a homemade-designed cell schematically shown in Figure 1. The respective polymer membrane was polymerized between two glass plates with a spacer of 0.2 mm, with the same ratio of monomers and cross-linker as in the immobilization experiment but without the enzyme. The resulting polymer was left in water overnight in the refrigerator. The membrane was placed between two chambers (Figure 1). To the upper chamber was introduced 0.8 mL of a triethanolamine 0.2 M buffer solution (pH = 7.8), which contained the substrate **7** ($1 \times 10^{-2}$ M). The same buffer solution without the substrate was allowed to flow through the lower chamber (30 $mL\cdot h^{-1}$). Fractions of the eluted solution were collected and analyzed spectroscopically for the content of **7** ($\lambda$ = 314 nm, $\epsilon$ = 10 000 $M^{-1}\cdot cm^{-1}$).

## Results and Discussion

The three photoisomerizable acrylamide copolymers 4-(methacryloylamino)azobenzene (**1**), 1-[β-methacyrloxy)ethyl]-3,3-dimethyl-6'-nitrospiro[indoline-2,2'-[2*H*-1]benzopyran] (**2**), and bis[4-(dimethylamino)phenyl](4-vinylphenyl)methyl leucohydroxide (**3**) exhibit reversible and cyclic photoisomerizable properties. Upon illumination, the *trans* form **1a** at 370 nm > λ > 330 nm, isomerizes to the *cis* form **1b**. Illumination of **1b** at λ > 400 nm results in the reverse isomerization and formation of **1a**. Illumination of copolymer **2a**, 400 nm > λ > 300 nm, results in the merocyanine copolymer **2b**. Illumination of the resulting red copolymer **2b**, λ > 475 nm, re-forms the original spiropyran copolymer **2a**. Copolymer **3a** undergoes heterolytic bond cleavage upon its illumination, 370 nm > λ > 330 nm, to form the green ionic copolymer **3b**. The latter copolymer undergoes thermal recombination to form **3a**. Figure 2 exemplifies the spectral changes associated with the cyclic photoisomerization of **1a** to **1b**. These photoinduced isomerization processes can be reversibly cycled for polymers **1–3** using the appropriate wavelengths or by a photochemical–thermal cycle for polymer **3**.

Photostimulated cyclic activation/deactivation of α-chymotrypsin immobilized in copolymer **1** with loading of the photo-

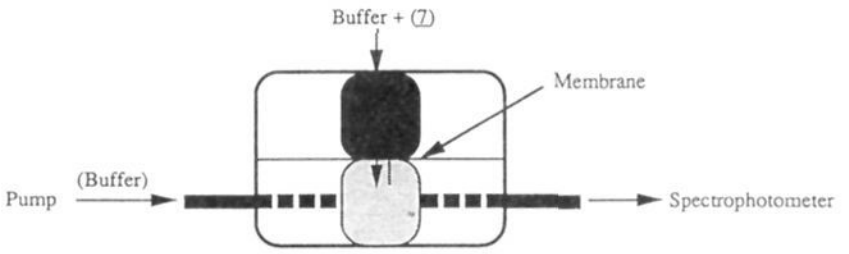



**Figure 1.** Flow-dialysis cell. The flow-dialysis cell has two chambers. The upper chamber contained the substrate **7** ($1 \times 10^{-2}$ M) in a buffer solution (triethanolamine 0.2 M, pH 3.8). In the lower chamber flowed the same buffer (30 $mL\cdot h^{-1}$). The photochromic copolymer was placed between the two chambers. The fractions of the eluted solution were collected and analyzed spectroscopically.

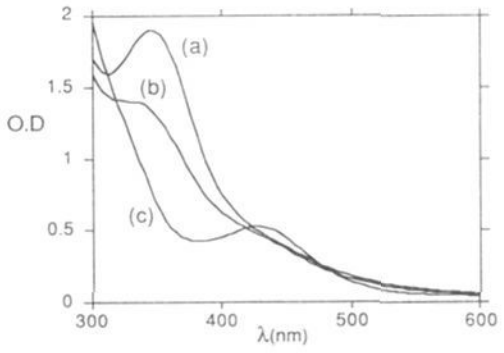
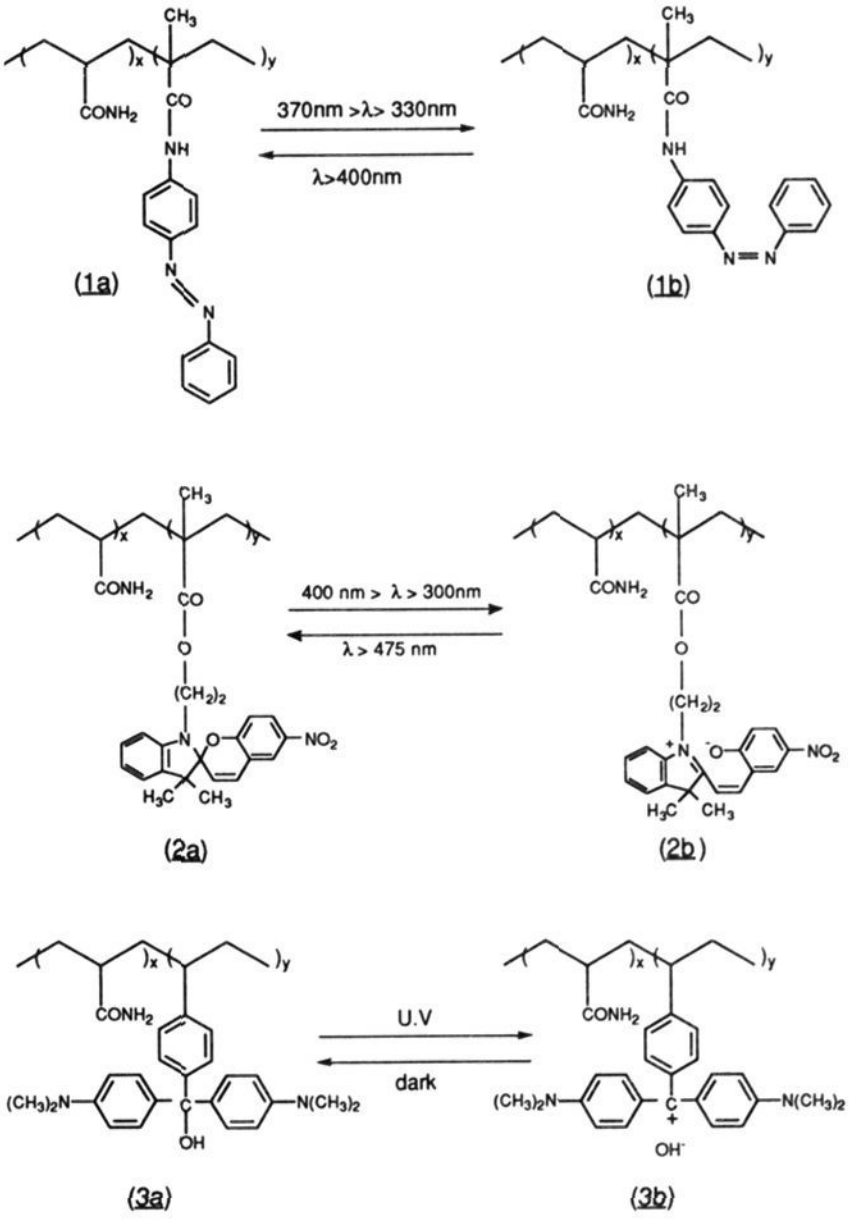



**Figure 2.** Photochromic attributes of azobenzene copolymer **1**: (a) 100% *trans*-**1** after irradiation, λ > 400 nm; (b) 50% *cis*-**1** after irradiation, 370 nm > λ > 330 nm; and (c) calculated spectrum of 100% *cis*-**1** according to Fischer's method (*J. Phys. Chem.* **1967**, *71*, 3704).

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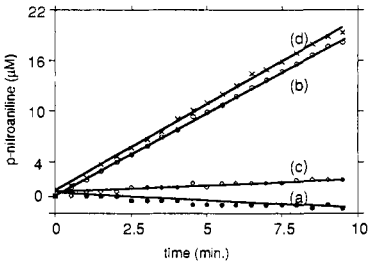
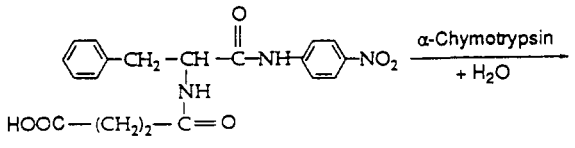
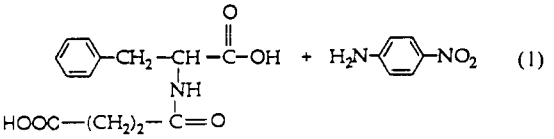



**Figure 3.** Rate of hydrolysis of **7** ($5.7 \times 10^{-3}$ M) by α-chymotrypsin (47.6 units) immobilized in copolymer **1** (loading 0.5 mol %): (a) hydrolysis by enzyme in the copolymer **1a** form; (b) after illumination of the copolymer, λ = 330–370 nm, and formation of copolymer **1b**; (c) after further illumination of the copolymer **1b**, λ > 400 nm, and regeneration of **1a**; and (d) after additional illumination, λ = 330–370 nm, and regeneration of **1b**. Experiments outlined in a–d are on the same polymer sample. Prior to each run, the polymer is washed with triethanolamine buffer, 0.2 M, pH = 7.8, and added to a new substrate solution to maintain the similar initial concentration of the substrate in all experiments.

isomerizable units corresponding to 0.5 mol % is shown in Figure 3. The enzyme activity is assayed toward the hydrolysis of *N*-(3-carboxypropionyl)-L-phenylalanine *p*-nitroanilide (**7**) (eq 1). It




(**7**)



(1)

can be seen that in the **1a** *trans* configuration of the polymer **6**, no biocatalyzed hydrolysis of **7** occurs (Figure 3a). Upon illumination of **1a**, 370 nm > λ > 330 nm, the copolymer undergoes structural isomerization to **1b** and the immobilized enzyme reveals hydrolytic activity. The rate of hydrolysis of **7** corresponds to $V_{max}$ = 2 μM·min$^{-1}$ (Figure 3b). Further illumination of the copolymer **1b**, λ > 400 nm for 1 h, restores the copolymer structure **1a**, and the entrapped enzyme is again deactivated (Figure 3c). The biocatalytic activity of the system can be further switched "on" by transforming the copolymer to structure **1b** as shown in Figure 3d, implying that the photoisomerizable polymer enzyme assembly exhibits cyclic and reversible photoswitchable activities. While the biocatalyst activity is switched "on" in the copolymer configuration **1b**, it is switched "off" in the copolymer isomer state **1a**. The switching efficiency that corresponds to the ratio of biocatalyst activities in the two photoisomer states of the copolymer strongly depends on the loading of the copolymer by the photoisomerizable units. Table I summarizes the rates of hydrolysis of **7** by α-chymotrypsin encapsulated in the copolymers in the two photoisomer states **1a** and **1b** as a function of the loading of the copolymer. For comparison, the rates of hydrolysis of **7** by α-chymotrypsin immobilized in an acrylamide polymer are also included. It is clear that up to a loading degree of 0.5 mol % of the copolymer by the photoisomerizable components, switchable activities of the entrapped enzyme are detected. At a higher loading, a low activity of the immobilized enzyme is observed in either the copolymer configuration **1a** or the copolymer state **1b**, presumably due to poor permeability of the substrate across the two isomer states of the copolymer. Furthermore, it is evident that up to a loading of 0.5 mol % of the copolymer by azobenzene units, the hydrolytic performance of the enzyme in the copolymer **1b** is higher than in a pure acrylamide polymer

**Table I.** Normalized Rates of Hydrolysis of **7** by α-Chymotrypsin Immobilized in the Azobenzene–Acrylamide Copolymer **1** at Different Degrees of Loading[a,b]

| | load = | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.18 | 0.3 | 0.5 | 0.75 | 1 |
| *v*(**1b**) | 2 | 5 | 4 | 4 | 1 | 1 |
| *v*(**1a**) | 2 | 2 | 1 | 0–0.3 | 1 | 1 |

[a] Loading is expressed in terms of mol % of the photoisomerizable component relative to the acrylamide monomer. [b] Rate of 1 corresponds to the formation of 0.5 μM·min$^{-1}$ of *p*-nitroaniline. Rate is expressed in μM·min$^{-1}$.

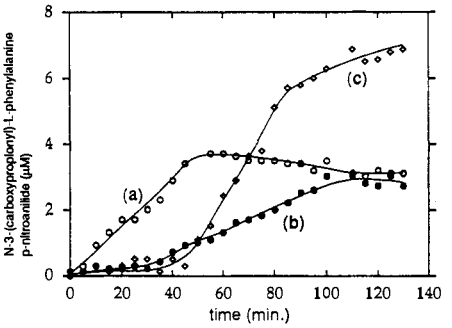



**Figure 4.** Flow dialysis of **7** through the azobenzene copolymer membrane: (a) flow dialysis of **7** through **1b**, loading 0.5 mol %; (b) flow dialysis of **7** through **1a**, loading 0.5 mol %; and (c) flow dialysis of **7** through **1b**, loading 1 mol %.

matrix. For example, the enzyme is ca. 2-fold more active in the copolymer **1b** as compared to in the acrylamide polymer system.

To account for the different activities of α-chymotrypsin in the two states of polymer environments, **1a** and **1b**, we examined the permeability of the reaction substrate **7** across the different photoisomer states of the polymer membranes. In these studies, flow dialysis was used to probe the permeabilities of the substrate **7** through the polymer membranes. In these experiments, the polymer is fabricated in the form of a flat membrane (1.5 cm in diameter) that separates two chambers. The substrate **7** is placed in the upper chamber, dialyzed across the membrane, and eluted from the lower chamber by a flow solution. (For details, see the Experimental Section.) Thus, the substrate eluted by the flow system as a function of time represents the permeability of the substrate through the respective membrane. Figure 4 shows the dialysis profiles of **7** through a polymer membrane consisting of **1a** and **1b**, respectively (loading 0.5 mol %). When the separating membrane is composed of **1b**, the substrate dialyzes immediately through the membrane and is detected in the eluted solution (Figure 4a). A characteristic flow-dialysis curve is observed, where the concentration of **7** increases with time (ca. 50 min) and reaches a steady-state value corresponding to the equilibrium between the eluted substrate and the dialyzing substrate. Interestingly, when **1a** is applied as the separating membrane in the flow-dialysis system, a different elution profile of the substrate is observed (Figure 4b). The dialysis of **7** across **1a** reveals an induction period of ca. 40 min. Afterward, **7** is detected in the eluted solvent, and the same steady-state dialysis value as for **1b** is observed after ca. 110 min. Thus, the uptake and the release of the substrate **7** by the copolymer membrane **1a** is less efficient as compared to that of the copolymer membrane **1a**. Examination of membrane permeability by flow-dialysis experiments is further demonstrated by using a membrane composed of 1 mol % of *cis*-azobenzene in the copolymer (Figure 4c). Here we see that even in the *cis* form of the photoisomerizable component, the permeability of the substrate across the copolymer **1a** is blocked for ca. 40 min.

We thus realize that the photoswitchable activities of the α-chymotrypsin entrapped in the azobenzene acrylamide copolymer correlates directly with the permeabilities of the respective

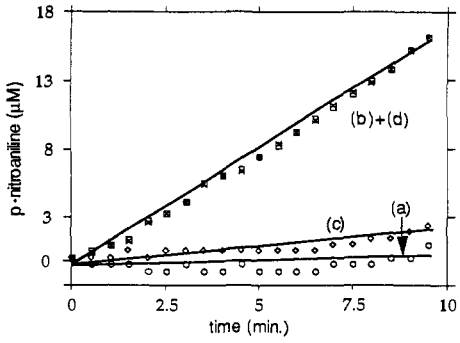



**Figure 5.** Rate of hydrolysis of **7** ($5.7 \times 10^{-3}$ M) by α-chymotrypsin (47.6 units) immobilized in copolymer **2** (0.12 mol %): (a) copolymer in the **2a** form; (b) copolymer in the **2b** state, formed upon illumination of **2a**, 400 nm > λ > 300 nm; (c) copolymer regenerated to **2a** by illumination, λ > 475 nm; and (d) copolymer recycled to the **2b** state by illumination.

polymer membranes toward the reaction substrate. In the poorly permeable copolymer structure **1a**, the immobilized biocatalyst is switched "off" toward the hydrolytic transformation, while in the permeable copolymer, configuration **1b**, the enzyme is switched "on" toward the hydrolysis of **7**. The activity of the enzyme in copolymer **1b** is higher than that in the pure acrylamide polymer where the permeability of **7** is lower.

Previous studies indicated that physical properties of azobenzene-modified copolymers can be photostimulated.[24] Isomerization of the *trans*-azobenzene chromophore to *cis*-azobenzene is accompanied by a structural change that involves an alteration of polarity. The distance between positions 4 and 4′ of azobenzene is shortened from a separation of 9 Å (*trans*) to 5.5 Å (*cis*),[25,26] and the dipole moment of the chromophore varies from 0.5 D (*trans*) to 3.1 D (*cis*).[24] These differences influence the bulk physical properties of the polymers and allow the design of photocontrolled physical phenomena in these assemblies. For example, the photostimulated transport of amino acids and carboxylic acids across graft copolymers that contain azobenzene units has been achieved.[27] Similarly, the sol–gel transitions of a polystyrene–azobenzene copolymer are controlled by light-stimulated *trans* → *cis* isomerization of the chromophore.[28] These two examples are consistent with the photoregulated permeabilities of **7** across the copolymer membranes **1a** and **1b**, which are discussed in the present study.

Similar "on–off" photostimulation of α-chymotrypsin is observed upon immobilization of the enzyme in the spiropyran acrylamide copolymer **2**. Figure 5 shows the rate of hydrolysis of **7** in a copolymer of 0.12 mol % of the spiropyran component. We see that the biocatalyst immobilized in the spiro configuration of the polymer **2a** is switched "off" and does not exhibit hydrolytic activity (Figure 5a). Upon illumination, 400 nm > λ > 300 nm for 2 h, the polymer is transformed into the zwitterionic-merocyanine state **2b** and the enzyme is activated toward hydrolysis of **7**, $V_{max} = 1.5\ \mu M \cdot min^{-1}$ (Figure 5b). Illumination of the biocatalyst–**2b**-polymer assembly, λ > 475 nm, restores the spiro polymer state **2a** and the enzyme is again blocked toward the hydrolytic transformation (Figure 5c). This photochemical stimulation of the enzyme can be further cycled by irradiation of the copolymer between the **2a** and **2b** states. The switching efficiency depends on the loading of the polymer by the spiropyran components. Table II summarizes the hydrolysis rates of the substrate **7** by α-chymotrypsin immobilized in the spiropyran acrylamide copolymers of different loadings and compares the biocatalyst activities to those of α-chymotrypsin immobilized in

**Table II.** Normalized Rates of Hydrolysis of **7** by α-Chymotrypsin Immobilized in the Spiropyran–Acrylamide Copolymer **2** at Different Degrees of Loading[a,b]

| | load = | | | |
|---|---|---|---|---|
| | 0 | 0.09 | 0.12 | 0.18 |
| *v*(**2b**) | 2 | 2 | 3 | 2 |
| *v*(**2a**) | 2 | 0.3 | 0–0.2 | 1 |

[a] Loading is expressed in terms of mol % of the photoisomerizable component relative to the acrylamide monomer. [b] Rate of 1 corresponds to the formation of 0.5 $\mu M \cdot min^{-1}$ of *p*-nitroaniline. Rate is expressed in $\mu M \cdot min^{-1}$.

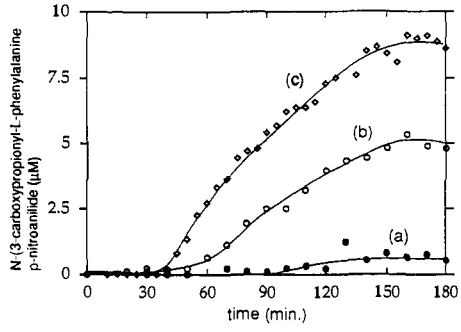



**Figure 6.** Flow dialysis of **7** through copolymer **2**: (a) membrane in the **2a** state; (b) membrane in the **2b** state formed after 1-h illumination of membrane **2a**, 400 nm > λ > 300 nm; and (c) membrane in the **2b** form generated by illumination of **2a** for 3 h, 400 nm > λ > 300 nm.

polyacrylamide. It is clear that the activity of α-chymotrypsin in the neutral spiroacrylamide copolymer **2a** strongly depends on the loading of the polymer by the photoisomerizable component. As the loading increases, the biocatalyst activity decreases (when the copolymer is in its spiro state) as compared to that in acrylamide, and at a loading degree of 0.12 mol %, the enzyme is switched "off". The activity of the enzyme in the zwitterionic-merocyanine state **2b** reveals, at a loading of 0.12 mol %, an enhancement in the hydrolytic activity as compared to that in the acrylamide polymer, but a further increase in the content of the spiropyran component (0.18 mol %) results in a decease in the biocatalyst activity. We thus realize that the highest switching efficiency is observed at the loading corresponding to 0.12 mol % of the photoactive component. Under these conditions, the enzyme is in a switched "off" state in the spiropyran polymer configuration **2a** and shows the highest activity in the merocyanine polymer **2b**.

The photostimulated activities of α-chymotrypsin immobilized in polymer **2** are also controlled by the permeabilities of the substrate across the polymer matrices. Figure 6 shows the corresponding flow-dialysis experiments for the different membrane forms at a loading of 0.12 mol %. It is clear that the neutral polymer **2a** is nonpermeable toward the substrate **7**, and after an induction time of ca. 100 min, only trace amounts of the substrate are dialyzed through the membrane (Figure 6a). Illumination of the **2a**-polymer membrane, 400 nm > λ > 300 nm, results in the transformation of the polymer to the **2b** state. The time of illumination of the membrane controls the permeability of the resulting membrane toward the substrate (Figure 6, curves b and c). It is evident that illumination of the membrane for 1 h results in the effective dialysis of the substrate after an induction period of 60 min. Illumination of the membrane for 3 h improved the permeability of the membrane, and dialysis of the substrate proceeded after 30 min.

Photoisomerization of the spiropyran copolymer **2a** is accompanied by significant changes in the physical properties of the polymer.[24] Under UV irradiation, spiropyran undergoes a ring opening leading to the formation of merocyanine which exhibits a high dipole moment. These structural and polarity changes of the photoactive components affect the structure of the polymer

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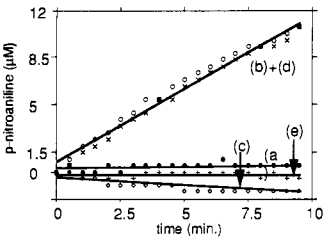


**Figure 7.** Rate of hydrolysis of **7** ($5.7 \times 10^{-3}$ M) by α-chymotrypsin (47.6 units) immobilized in copolymer **3** (0.2 mol %): (a) copolymer in the **3a** state; (b) after illumination of the membrane with UV light and conversion of the membrane to **3b**; (c and e) copolymer restored to the **3a** state; and (d) copolymer in its **3b** state.

**Table III.** Normalized Rates of Hydrolysis of **7** by α-Chymotrypsin Immobilized in the Triphenylmethane Leucohydroxide Copolymer **3** at Different Degrees of Loading[a,b]

| | load = | | |
|---|---|---|---|
| | 0 | 0.2 | 0.4 |
| $v$(**3b**) | 2 | 2 | 1 |
| $v$(**3a**) | 2 | 0 | 1 |

[a] Loading is expressed in terms of mol % of the photoisomerizable component relative to the acrylamide monomer. [b] Rate of 1 corresponds to the formation of 0.5 μM·min$^{-1}$ of *p*-nitroaniline. Rate is expressed in μM·min$^{-1}$.

or membrane. For example, poly-L-glutamic acid modified by spiropyran exhibits a random coil structure in its merocyanine state and undergoes a structural organization to an α-helix form upon electrocyclization of the photoactive components to the spiropyran state.[29]

The effect of the photoisomerization of spiropyran to merocyanine on the electrical potential of surfaces was similarly revealed by Langmuir–Blodgett films.[30] It was shown that the electrical potential of such spiropyran monolayers is positively shifted upon photoisomerization to the merocyanine monolayer configuration. Thus, photoisomerization of spiropyran-modified polymers or interfaces to the merocyanine isomer stimulates structural and electrical changes in these macromolecular assemblies. Control of the permeabilities of the substrate **7** across the polymer membranes **2a** and **2b** could then originate from structural and electrical modification of the membranes.

Another "on–off" photoregulated enzyme–polymer assembly is organized upon encapsulation of α-chymotrypsin in the copolymer **3**. Figure 7 shows the rate of hydrolysis of **7** by α-chymotrypsin immobilized in **3a** or **3b** that contains 0.2 mol % of bis[4-(dimethylamino)phenyl](4–vinylphenyl)methyl leucohydroxide as photoactive groups. As in the two previous systems, the hydrolysis of **7** is fully controlled. In Figure 7a, the copolymer is in the **3a** form and the enzyme–copolymer assembly does not exhibit hydrolysis activity. Upon UV illumination, $370 > \lambda > 330$ nm, the copolymer is transformed to the **3b** form and the enzyme is activated toward hydrolysis of **7**, $V_{max} = 1$ μM·min$^{-1}$ (Figure 7b). Keeping the copolymer in the dark for 2 h restored it to its neutral form (**3a**), and the resulting enzyme–polymer assembly did not reveal any hydrolytic activity (Figure 7c). Curves 7d and 7e demonstrate the reversible cycling of the activities of α-chymotrypsin by a second photoactivation of the polymer to the form **3b** followed by the dark isomerization of the polymer to the **3a** form. Table III summarizes the hydrolysis rates of **7** by α-chymotrypsin immobilized in assemblies of polymer **3** of different loadings. Only the loading of 0.2 mol % of the photoactive groups reveals the system-photocontrolled activities. Flow-dialysis experiments that examine the permeability changes

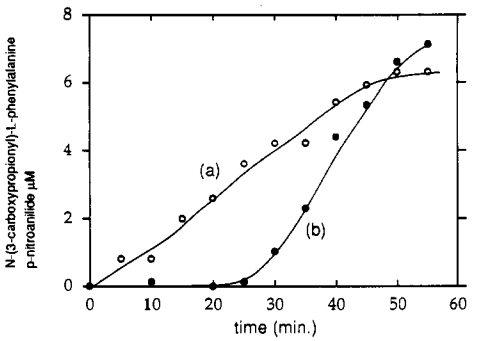


**Figure 8.** Flow dialysis of **7** through copolymer **3**: (a) membrane in the **3b** state and (b) membrane in the **3a** state.

of **7** across the photoswitchable film **3a** and **3b** at a loading of 0.2 mol % are shown in Figure 8. The flow dialysis across the membrane consisting of the neutral copolymer **3a** is shown in Figure 8a. This membrane shows no permeability toward the substrate **7** for the first 25 min of the experiment. The membrane in the **3b** form shows, however, immediate permeability toward **7**. Previous studies have indeed indicated that photoisomerization of copolymers that include bis[4-(dimethylamino)phenyl](4-vinylphenyl)methyl leucohydroxide as photoactive groups exhibit light-stimulated physical properties. For example, a copolymer in a gel form has revealed photoregulated volume-phase transitions.[31] We reveal in the present study that permeability of the substrate **7** across the copolymer configurations **3a** and **3b** is controlled by light.

## Conclusions

We have described the cyclic and reversible photoregulation of α-chymotrypsin activity in a series of photoisomerizable copolymers. In contrast to previous attempts to photoregulate proteins by direct interaction of the photoisomerizable units to proteins, where only partial deactivation of enzymes was observed, the present systems represent "full photoswitches", that is, the "off" position of the enzyme–polymer assembly represents zero activity. In the present study, we reveal that the photoswitchable activities of α-chymotrypsin in these polymers originate from photocontrolled permeability of the biocatalyst substrate across the polymer membranes. In the series of the three polymers, we see that substrate **7** is permeable across the *cis*-azobenzene copolymer **1b**, the zwitterionic-merocyanine copolymer **2b**, and the heterolytically-dissociated leucohydroxide copolymer **3b**. The most likely explanation for the enhanced permeabilities of **7** across the polymer forms **1b**, **2b**, and **3b** is the increased polarity of these polymer matrices that favors the transport of the polar and charged substrate. The dipole moment of the *cis*-azobenzene units in **1b** (3.1 D) is higher than that for the *trans*-azobenzene components in **1a** (0.5 D).[24] The electrocyclic opening of **2a** to the zwitterionic-merocyanine structure **2b** yields a polar environment. Similarly, the cationic form of copolymer **3b** exhibits higher polarity as compared to the neutral form of copolymer **3a**. Hydration of these highly polarized polymers might increase the water content in the polymers and their macroscopic volumes. These effects together with electrostatic interaction facilitate the transport of the substrate through these polymer membranes. Nonetheless, the polymers applied in this study to photoregulate α-chymotrypsin are known to fatigue. We estimate that ca. 100–200 cycles of enzyme-switchable activities can be achieved prior to fatiguing of the polymers.

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