Dissociative Electron Capture. The experimental results obtained in the present study clearly establish the propensity of sulfone and sulfonate molecules to undergo a dissociative electron-capture process. Poly(olefin sulfones), having a higher proportion of the sulfone group, are expected to be more vulnerable to such a process. The present result does not rule out the mechanism suggested earlier for the radiolysis of these materials; it does give credence to the new postulate that the primary step in the radiolysis of these materials might be dissociative electron capture. Absence of the ESR signals due to sulfonyl radicals $RSO_2^{\bullet}$ in samples irradiated at low temperature may be ascribed to the process where the sulfonyl end remains as an anion (RSO_2^-). Emergence of the ESR signals due to sulfonyl radicals on warming may be attributed to thermal release of trapped electrons and/or holes and resulting electron hole recombination. Isomerization observed in the olefinic monomers released in the radiolysis of poly(olefin sulfones) may have occurred during depropagation of polymer chains having ionized sectors.

The Na to sulfone (or sulfonate) electron transfer actually effected was so facile that the majority of the reaction occurred during deposition through collision in space or in a fluid surface layer of the developing matrix. Tetramethylene sulfone was an exception in that no electron transfer was observed prior to photoirradiation. The exception, we believe, is due to a lower sample pressure possible from tetramethylene sulfone and to a steric factor unfavorable for the collision process.

In an effort to gain further insight to the process involved, molecular orbital calculations (EHT) were performed. The calculations showed that the LUMO (lowest unoccupied orbital) in the cases of dimethyl sulfone and tetramethylene sulfone would be an antibonding σ orbital confined to the CSC sector. For methyl methanesulfonate and methyl trifluoromethanesulfonate, the predicted LUMO was an antibonding σ orbital confined to the CO sector. In methyl methanesulfonate, the second lowest unoccupied orbital was shown to be an antibonding σ orbital confined to the CS sector. The corresponding orbital in the methyl trifluoromethanesulfonate was much higher in energy as expected. The predicted energy levels of these orbitals are such that the stable anions (of the original structure) would not be formed. The dissociative electron capture of sulfones and sulfonates must then occur through repulsive negative ion resonance states, and the point of scission is determined by the electronegativity of the SO_2 or SO_3 moiety and weakness of the C-S and C-O bonds.

(25) Kasai, P. H. *J. Am. Chem. Soc.* **1990**, *112*, 4313.

Photoregulation of Papain Activity through Anchoring Photochromic Azo Groups to the Enzyme Backbone

Itamar Willner,* Shai Rubin, and Azalia Riklin

Contribution from the Department of Organic Chemistry, The Hebrew Univeristy of Jerusalem, Jerusalem 91904, Israel. *Received January 22, 1990. Revised Manuscript Received August 21, 1990*

Abstract: The enzyme papain has been chemically modified by 4-carboxyazobenzene (**1**), 3-carboxyazobenzene, (**2**), and 2-carboxyazobenzene, (**3**). The activities of the modified enzymes relative to native papain and the extent of loading by the azobenzene groups have been determined. *trans*-**1**-papain exhibits reversible photochromic properties, and upon illumination (λ = 320 nm), interconversion to *cis*-**1**-papain occurs. Further illumination of *cis*-**1**-papain (λ > 400 nm) regenerates *trans*-**1**-papain. The biocatalyst *trans*-**1**-papain is 2.75-fold more active than *cis*-**1**-papain toward hydrolysis of N^{α}-benzoyl-DL-arginine-4-nitroanilide (BAPNA; **4**). The difference in activities of the trans/cis photochromic enzyme allows photoregulation of the hydrolytic process. Kinetic analyses reveal that the difference in activities of the two photochromic forms of **1**-papain originates from poorer binding properties of *cis*-**1**-papain toward the substrate, as compared to *trans*-**1**-papain. The biocatalyst *trans*-**1**-papain is immobilized into Alginate beads, and cyclic photoregulated hydrolysis of BAPNA is effected.

Photoregulation of enzyme activities provides a general approach to utilize biocatalysts as light-controlled "on-off" switching systems. Potential applications of photoregulated enzymes include their use as light signal amplifiers, information storage devices, and sensoring assemblies.[1,2] Photoregulation of enzymes has been previously described by using photochromic materials that act as inhibitors for the enzyme active site specifically in one of their photochromic configurations.[3,4] Also, chemical derivatization of the enzyme active site by photochromic substituents is capable of blocking the biocatalyst activity in one of the photochromic structures.[5] Other approaches to photoregulate enzymes involve chemical modification of the biocatalyst active site with photoactive groups deactivating the enzyme, but upon illumination, the blocking group is released and consequently the biocatalyst activity is restored.[6] In a single report,[7] chemical modification of the protein backbone of enzymes by the spiropyran photochromic material led to photoregulation of a few enzymes. The photoregulative activities of these enzymes have been attributed to alterations in hydrophilicity-hydrophobicity of the protein microenvironments resulting from the neutral-zwitterionic photochromic forms of spiropyran. Nevertheless, comparison of the chemically modified enzyme activities to the native catalysts is scarce. Also, in all of the reported photoregulated biocatalytic assemblies no cyclic "on-off" switching performance is documented. Here, we wish to report on photoregulation of papain by anchoring azobenzene photochromic groups to the protein backbone. Azobenzene undergoes photoinduced trans to cis

(1) Hug, D. *Photochem. Photobiol. Rev.* **1978**, *3*, 1-33.
(2) Martinek, K.; Berezin, I. V. *Photochem. Photobiol.* **1979**, *29*, 637-649.
(3) Bieth, J.; Vratsanos, S. M.; Wassermann, N. H.; Erlanger, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *64*, 1103.
(4) Wainberg, M. A.; Erlanger, B. F. *Biochemistry* **1971**, *10*, 3816.
(5) (a) Erlanger, B. F.; Wassermann, N. H.; Copper, A. G.; Monk, R. J. *Eur. J. Biochem.* **1976**, *61*, 287. (b) Montagnoli, G.; Balestreri, E.; Nannicini, L.; Bellucci, A.; Bracaloni, M. *Int. J. Pept. Protein Res.* **1978**, *11*, 28.
(6) Turner, A. D.; Izzo, S. V.; Rozakis, G. W.; Porter, N. A. *J. Am. Chem. Soc.* **1987**, *109*, 1274-1275.
(7) Aizawa, M.; Namba, K.; Suzuki, S. *Arch. Biochem. Biophys.* **1977**, *182*, 305-310.

0002-7863/91/1513-3321$02.50/0

Table I. Loading Degrees and Activities of *cis*- and *trans*-Azobenzene–Papains (Compared to Native Papain)

enzyme	loading[a]	activity[b] (%)
papain	0	100
trans-**1**–papain	4.6	80
cis-**1**–papain	4.6	30
trans-**1**–papain	3.7	80
cis-**1**–papain	3.7	53
trans-**2**–papain	5.2	36
cis-**2**–papain	5.2	21
trans-**3**–papain	5.3	1
cis-**3**–papain	5.3	1

[a] Loading is defined as number of dye molecules/protein backbone. [b] Activity is defined (rate of modified enzyme/rate of native enzyme) × 100. Rates are determined by assay described in the text.

isomerization being accompanied by a substantial increase in the molecular dipole moment (from 0 to 3.0 D) and significant steric change (from a planar to a nonplanar structure).[8] We discuss the effects of chemical modification of the protein by the photochromic materials on the activities of the enzyme. Also, photoregulation of papain through trans to cis isomerization of photochromic substituents is exemplified.

Experimental Section

Azobenzene-4-carboxylic acid, (**1**),[9] azobenzene-3-carboxylic acid (**2**),[9] and azobenzene-2-carboxylic acid (**3**)[10] were prepared according to the literature. All other chemicals are from commercial sources (Aldrich and Sigma). Dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) must be dry. NHS is purified and dried by recrystallization from ethyl acetate (1 g/6 mL) until a melting point of 99–100 °C is obtained. THF is dried over sodium.

A general procedure for modification of lysine residues of papain by azobenzenecarboxylic acids has been applied.[11] All reactions are performed under nitrogen in a glovebox. DCC (0.3 mmol) is dissolved in THF (1 mL), and the solution is cooled to 0 °C. The DCC solution is added to a cold (O °C) THF solution (2 mL) that includes the respective azobenzenecarboxylic acid (0.3 mmol) and NHS (0.3 mmol). The resulting mixture is allowed to react for 2 h (0 °C) and left overnight in the refrigerator. The active ester solution is filtered into a cooled aqueous solution (12 mL) that includes 0.5 g of sodium bicarbonate and 120 mg of papain. The resulting mixture is left to react under nitrogen at 0 °C for 24 h. The white turbidity formed is centrifuged (4500 rpm), and the supernatant is dialyzed against water/THF (10:1) (three times) and against pure water (six times). The dialyzed solution is centrifuged again (30 min, 4 °C, 15 000 rpm), and the supernatant is lyophilized to obtain a powder of the modified papain.

Papain and azobenzene-modified papains are assayed by following the rate of hydrolysis of N^{α}-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA; **4**)[12] and by following the formed *p*-nitroaniline using HPLC. The general assaying procedure of modified papains includes dissolution of the enzyme (0.5 mg in 0.6 mL of water) and illumination of the solution (320 or >400 nm) to obtain *cis*- or *trans*-azobenzene–papains, respectively. The enzyme is added to a BAPNA substrate solution (3 mL, 1×10^{-3} M), and 50-μL samples of the reaction mixtures are withdrawn every 5 min and analyzed by HPLC.

Immobilization of azobenzene–papain in Alignate beads[13] is performed by preparing a mixture of 0.5 mg of the enyzme in 0.5 mL of Tris buffer solution (pH = 7.5) and 1 mL of alginate solution (3%). The resulting solution is then slowly dropped into 2 mL of $CaCl_2$ solution (0.1 M). The resulting beads including entrapped enzyme are centrifuged and separated. For continuous "on–off" switching of the enzyme–alginate assembly, the beads are introduced into a BAPNA solution (1×10^{-3} M, 3 mL) and the rate of hydrolysis is determined under steady-state illumination of the specified wavelengths. After each on–off cycle, the Alginate beads are separated by centrifugation and a fresh BAPNA solution is added.

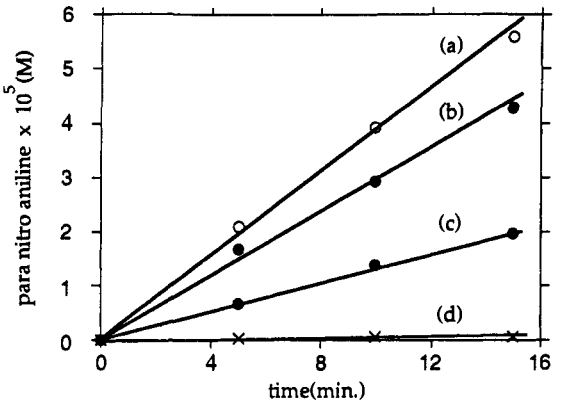
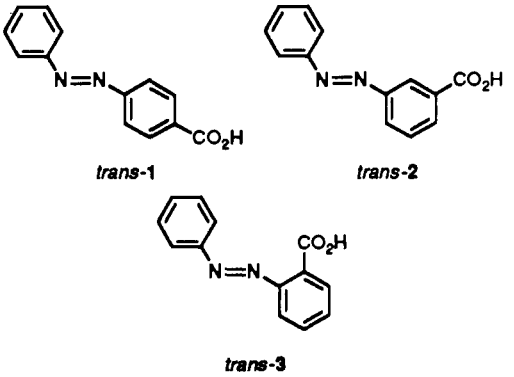


Figure 1. Rate of hydrolysis of BAPNA (**4**) (a) by native papain, (b) by *trans*-**1**–papain, (c) by *trans*-**2**–papain, and (d) by *trans*-**3**–papain. All systems are composed of an aqueous solution (3.6 mL, pH = 7.5) that includes **4**, 8.3×10^{-4} M and the respective photochromic enzyme (0.5 mg) with the maximum loading (Table I).

Results and Discussion

Papain has been modified by *trans*-azobenzene-4-carboxylic acid (**1**), *trans*-azobenzene-3-carboxylic acid (**2**), and *trans*-azobenzene-2-carboxylic acid (**3**) through anchoring the photochromic materials to lysine protein residues by an amide linkage.[14] The



extent of the resulting loading of papain by the different *trans*-azobenzenes, where 0.3 mmol of the dyes are reacted with 120 mg of papain (see experimental section), are provided in Table I. The loading degree has been determined by using the extinction coefficients of the respective *trans*-azobenzene, (ϵ(**1**)(322 nm) = 26 600 M^{-1} cm^{-1}; ϵ(**2**)(314 nm) = 18 200 M^{-1} cm^{-1}; ϵ(**3**)(316 nm) = 21 100 M^{-1} cm^{-1}) and determination of the protein content by the microprotein determination method (Sigma). A complementary method for estimating the loading degree has applied fluorescamine staining of the protein. Fluorescamine selectively binds to free lysine residues of the protein, and the resulting dye–protein complex fluoresces at λ = 475 nm (excitation λ = 390 nm). For example, the fluorescence intensity of fluorescamine-stained *trans*-**1**–papain corresponds to 54% of the fluorescence intensity of the fluorescamine-stained native papain. Since the protein backbone of papain includes ten lysine residues and additional terminal amino groups, the fluorescamine method implies an average degree of loading of 5 units of *trans*-**1** on the protein backbone. Furthermore, it is evident that good agreement of the loading degree of the protein is obtained by the two methods.

The biocatalytic activities of azobenzene-modified papains in comparison to the activity of the native enzyme are displayed in Figure 1. It is evident that *trans*-4-carboxyazobenzene–papain exhibits ca. 80% of the activity of the native enzyme, while *trans*-3-carboxyazobenzene–papain has 36% of the papain activity and *trans*-2-carboxyazobenzene–papain is deactivated upon the

(8) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915–1925.
(9) Anspon, H. D. *Org. Synth.* **1946**, *25*, 86.
(10) Freundler, M. P. *Bull. Soc. Chim. Fr.* **1807**, *1*, 219.
(11) Gary, E. M.; Feeney, R. E. *Chemical modification of proteins*; Holden-Day: San Francisco, 1971.
(12) Arnon, R. In *Methods in Enzymology*; Perlmann, G. E., Lorand, L., Eds.; Academic Press: New York and London, 1970; Vol. 19, p 226.
(13) Alginate is a commercial immobilization material. Protan Biopolymers, Drammen-Norway. Cf.: (a) Brodelius, P.; Deus, B.; Mosbach and Zenk, M. H. *FEBS Lett.* **1979**, *103*, 93. (b) Lim, F.; Sun, A. M. *Science* **1980**, *210*, 908–910.
(14) For general photochromic properties of azo compounds, cf.: (a) Fischer, E. *Forstchr. Chem. Forsch.* **1966/7**, 7, 605. (b) *Techniques of Chemistry*; Weissberger, A., Series Ed.; Vol. 3; Photochromism; Brown, G. H., Ed.; Wiley-Interscience: New York, 1971.

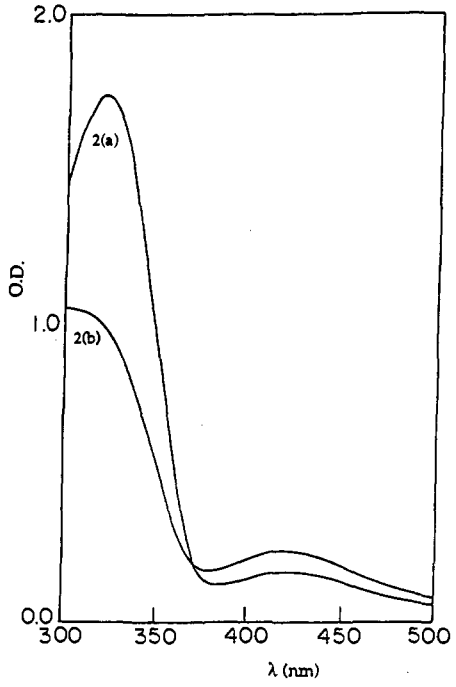
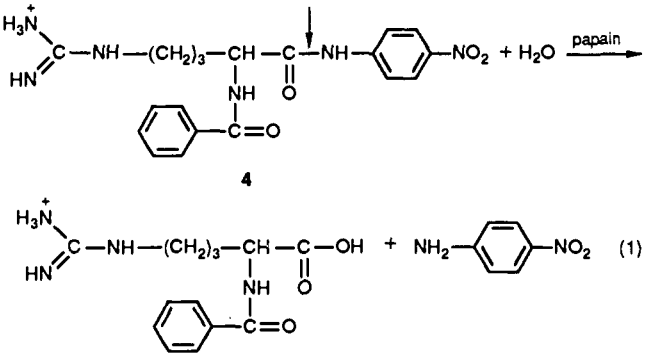


Figure 2. Photochromic properties of **1**-papain: (a) absorption spectrum of *trans*-**1**-papain; (b) absorption spectrum of *cis*-**1**-papain.

chemical modification process. The activity of the azobenzene-modified papains is unchanged for weeks in a powdered form (0 °C) and for at least 3 days in an aqueous solution at room temperature, implying that the enzymes exhibit high stabilities. The activities of papain- and azobenzene-modified papains have been determined by assaying the hydrolysis of N^{α}-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA; **4**; eq 1).



The azobenzene-modified papains exhibit photochromic properties. For example, illumination of *trans*-4-carboxyazobenzene–papain in an aqueous solution (pH = 7.5; λ = 320 nm; Figure 2a) results in the depletion of the absorption band at λ_{max} = 322 nm and the increase of the absorption band at λ_{max} = 424 nm (Figure 2b), corresponding to *cis*-**1**-derivatized papain. Further illumination of *cis*-**1**-modified papain (λ > 400 nm) restores the absorption band at λ_{max} = 322 nm and depletes the band at λ = 424 nm, implying that *trans*-**1**-papain is regenerated and that the modified *trans*-**1**-papain exhibits reversible photochromic properties. It should be noted that the absorption band at 322 nm, characteristic of *trans*-azobenzene, is not entirely depleted, and no further change in the absorption spectrum shown in Figure 2b is observed upon further illumination at λ = 320 nm. This result implies that interconversion of *trans*-**1**-papain to *cis*-**1**-papain corresponds, in fact, to a photostationary equilibrium. 4-Carboxyazobenzene (**1**) exhibits similar photochromic properties to those observed with the **1**-modified enzyme. Illumination of *trans*-**1** at 320 nm results in a photostationary equilibrium of *cis*- and *trans*-**1** exhibiting similar absorbance spectra observed for the modified papain. HPLC analysis of the *cis*/*trans*-**1** photostationary equilibrium mixture reveals that [*cis*-**1**]/[*trans*-**1**] corresponds to 0.89. Thus, we assume that a similar photostationary equilibrium is established in the photochromic papain. Thus, on average, 47% of the *trans*-azobenzene groups anchored to the protein backbone are interconverted into *cis*-azobenzene moities. The activities of the photogenerated *cis*-azobenzene-modified papains were determined (eq 1). The activities of the *cis*-azobenzene–papains in comparison to the parent *trans*-azobenzene–papains and the native enzyme are also included in Table I. Figure 3 exemplifies the rate of hydrolysis of BAPNA (**4**) by *trans*-**1**-papain and the photogenerated *cis*-**1**-papain. It is evident that *trans*-**1**-papain is ca. 2.75-fold more active than the isomerized functionalized protein, *cis*-**1**-papain. It should be noted that the rate of *cis*-**1**-papain corresponds, in fact, to the rate of a biocatalyst where only 47% of the azo groups are isomerized into the cis configuration and the rest are present in a trans structure.

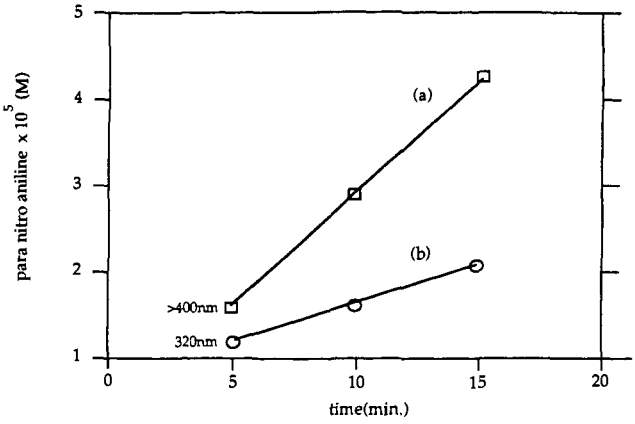


Figure 3. Rate of hydrolysis of BAPNA (**4**) (a) by *trans*-**1**-papain and (b) by *cis*-**1**-papain. The system is composed of an aqueous solution (3.6 mL, pH = 7.5 mL) that includes **4**, (8.3×10^{-4} M) and the photochromic enzyme (0.5 mg; dye loading, 0.05 mg dye/1 mg protein, corresponding to a loading degree of 4.6).

The differences in the biocatalytic activities of photochromic azobenzene-modified papains allows us to photoregulate the enzyme by an external light source. Figure 4 shows the sequential on–off and off–on switching of **1**-modified papain. In Figure 4a, hydrolysis of **4** is initiated by *trans*-**1**-papain, and after 15 min of reaction, the system is illuminated at λ = 320 nm (arrow). Interconversion of *trans*-**1**-papain to *cis*-**1**-papain occurs, and simultaneously, the rate of hydrolysis is slowed down as compared to the starting rate. Similarly, the hydrolysis rate can be switched on as shown in Figure 4b. Here, the substrate **4** is added to the prephotochemically generated solution of *cis*-**1**-papain, and a slow hydrolysis rate is observed for ca. 15 min. After this time, the system is illuminated (λ > 400 nm) and *trans*-**1**-papain is restored. Regeneration of the *trans*-**1**-papain is accompanied by an acceleration of the hydrolysis rate. We thus conclude that anchoring photochromic azobenzene substituents can be utilized to photoregulate the activity of papain. Cyclic photoregulated switching of **1**-modified papain involves difficulties since the absorbance of the hydrolysis product, *p*-nitroaniline, interferes with the light induced trans → cis isomerization reactions. To overcome this difficulty and allow cyclic photoregulation of the photochromic papain, *trans*-**1**-papain has been immobilized in Alginate beads. Immobilization of the photochromic biocatalyst in the transparent beads allows cyclic on–off operations of the system through separation of the reaction medium (product) from the immobilized biocatalyst and substitution of the system by a fresh substrate solution after each switching cycle. Using this technique, we were able to drive five on–off cycles with immobilized *trans*-**1**-papain with no noticeable degradation of the biocatalyst photoregulative activity.

It should be noted, however, that only a moderate difference in activities of the photochromic biocatalyst is observed as a result of structural isomerization of the anchored groups. Yet, with this specific photochromic biocatalyst, a stationary cis/trans equilibrium is established, and in fact, in the *cis*-**1**-papain ca. 53% of

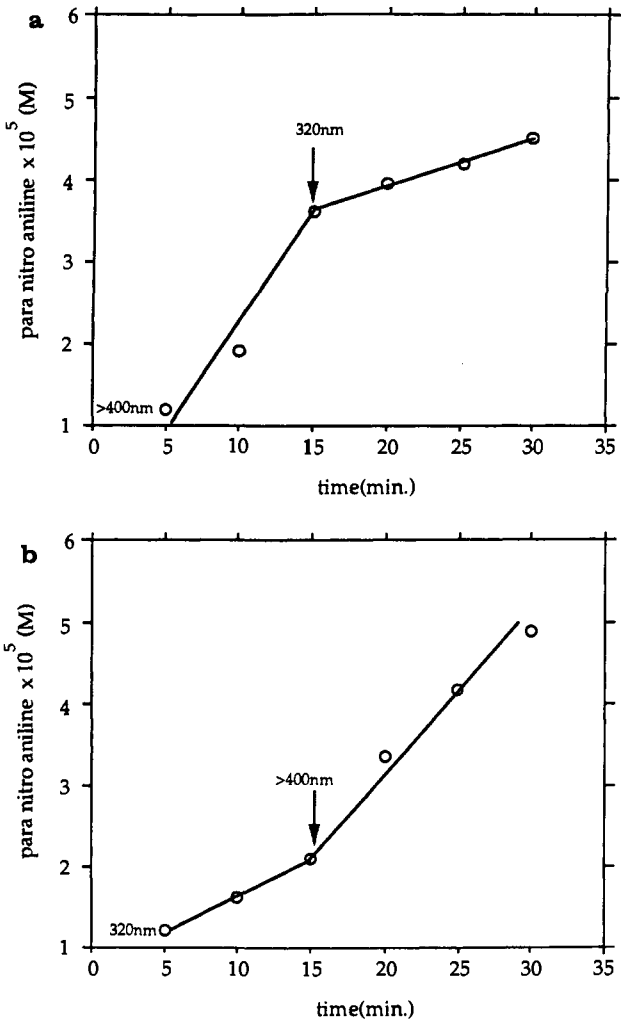


Figure 4. Photoregulated hydrolysis of BAPNA (4). (a) Hydrolysis is initiated by *trans*-1-papain and switched off by *cis*-1-papain. The arrow indicates photochemical conversion of *trans*-1-papain to *cis*-1-papain. (b) Hydrolysis is initiated by *cis*-1-papain and then accelerated by *trans*-1-papain. The arrow indicates photoinduced conversion of *cis*-1-papain to *trans*-1-papain. The system is composed of an aqueous solution (3.6 mL, pH = 7.5) that includes 4 (8.3×10^{-4} M) and the photochromic enzyme (0.5 mg; dye loading, 0.05 mg dye/1 mg protein, corresponding to a loading degree of 4.6).

the anchored photochromic groups are still present in the trans configuration. The results imply that isomerization of *trans*-azobenzene substituents to the *cis*-azobenzene configuration results in deactivating effects on the protein activity. Thus, selection of photochromic materials where trans → cis conversion is favored is anticipated to increase the differences in activities of the photochromic papains and to favor selective photoregulation.

Isomerization of the *trans*-azobenzene-anchored groups to *cis*-1-papain results in a deactivation of the biocatalyst toward hydrolysis. The deactivation phenomenon could originate from poorer binding properties toward the substrate, from perturbations in the catalytic function of the active site, or from both effects operating together. One can distinguish between the modes of deactivation of *cis*-1-papain by a kinetic analysis[15] of 1-papain in the trans and cis configurations. The Linweaver-Burk plots of *trans*-1-papain and *cis*-1-papain reveal that the two biocatalysts exhibit a similar V_{max} value (for an enzyme concentration of 2 μM), $V_{max} = 1.9 \pm 0.2$ μM min^{-1}, but differ substantially in their K_m values (K_m(trans) = 2.2 ± 0.2 mM; K_m(cis) = 6.5 ± 0.6 mM). Such kinetic behavior of enzymes is characteristic of a competitive

(15) (a) Fersth, A. *Enzyme Structure and Mechanism*; W. H. Freeman: Reading and San Francisco, 1977. (b) Roberts, D. V. *Enzyme Kinetics*; Cambridge University Press: Cambridge, 1977.

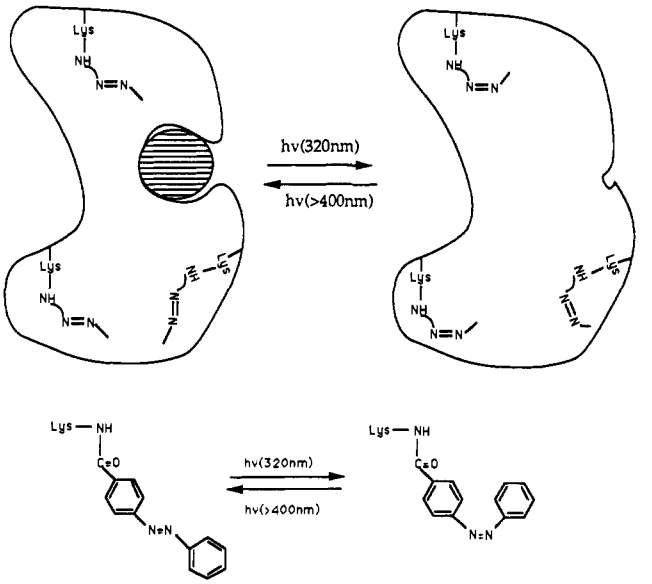


Figure 5. Schematic structural changes of the protein backbone through photochemical isomerization of anchored groups exerting a remote effect on the binding properties of the biocatalyst toward the substrate.

inhibition process where the inhibitor binds to a protein site different from the active site. This remote association of the inhibitor affects the binding properties of the substrate to the active site but not the catalytic functions of the active site. Thus, one can consider the different activities of *trans*-1-papain isomerization to *cis*-1-papain in terms of competitive inhibition. Namely, isomerization of the trans photochromic groups into the cis structure results in an inhibition effect toward binding of the substrate, while the catalytic functions of the cis biocatalyst are not affected. Thus, isomerization of the trans photochromic-anchored groups into the cis substitutents results in a structural change in the protein backbone that reduces the binding properties of the active site toward the substrate (Figure 5).

Some insight into the origin of the photoregulation effects on papain activity through anchoring 1 to the protein backbone can be derived from complementary experiments. The difference in activities of *trans* and *cis*-1-papains strongly depends on the extent of loading of the enzyme by photochromic groups. Decrease of the dye loading to 3.75×10^{-3} mg dye/1 mg protein, corresponding to the average substitution of 2.9 dye groups on the protein backbone, results in a sharp decrease in the difference in activities of *trans*-1-papain and *cis*-1-papain, and the two forms exhibit almost similar activities. Thus, the extent of loading of the protein backbone by the photochromic components strongly affects its photoregulative properties.

Attempts to increase the degree of loading of the photochromic groups above the average value of 4.6 groups/protein failed. The structure of papain has been elucidated, and the protein backbone includes 10 lysine residues. Among these groups, 4–6 residues are peripherally located on the protein structures,[12,16] while the others are internally shielded. Furthermore, no lysine residue participates in the active-site structure. These considerations suggest that chemical modification of papain by 1 results in primary derivatization of peripheral lysine residues and that the photoregulation effects originate from structural changes in the protein backbone exerting a remote effect on the active-site binding capability toward the substrate. A schematic representation of the photochromic groups functions in photoregulating the biocatalyst is provided in Figure 5. This speculative interpretation certainly needs further support. Examination of the enzyme purity of substitution, and sequencing of the enzymes for elucidating the positions of substitution and the photoisomerized sites, could provide further information on the photoregulation phenomenon.

(16) Gray, C. J. *Enzyme Catalysed Reactions*; Van Nostrand Reinhold: London, 1971.

Attempts to perform these complex sequencing experiments are now being examined.

In conclusion, chemical modification of the protein backbone of papain by azobenzene photochromic groups allows photoregulation of the enzyme. Photoinduced isomerization of *trans*-azobenzene groups to *cis*-azobenzene is probably accompanied by structural changes in the protein backbone that affect the binding properties of the enzyme toward the substrate. Further studies that include chemical modification of the enzyme by other photochromic materials and structural characterization of **1**-modified papain are being examined in our laboratory.

Acknowledgment. The support of the Niedersachsen Land Foundation, FRG, is gratefully acknowledged.

Registry No. 1, 37790-20-8; **2**, 37790-19-5; **3**, 131618-74-1; **4**, 37039-24-0; papain, 9001-73-4.

Luminescence Probe and Voltammetry Study of Ion Transport during Redox Switching of Poly(pyrrole) Thin Films

V. Krishna, Y.-H. Ho, S. Basak, and K. Rajeshwar*

Contribution from the Department of Chemistry, Box 19065, The University of Texas at Arlington, Arlington, Texas 76019-0065. *Received August 6, 1990*

Abstract: The ion transport accompanying the redox switching of poly(pyrrole) thin-film electrodes was studied in situ by a new luminescence probe technique. Counterions such as pyrene sulfonate and naphthalene sulfonate, which are fluorescent yet electrochemically silent over the potential window of interest, were used for this purpose. The anodically synthesized poly(pyrrole)-pyrene sulfonate (PP/PS) and poly(pyrrole)-naphthalene sulfonate (PP/NS) thin films were first characterized by cyclic voltammetry. Distinctly different voltammetric fingerprints with associated memory effects were registered for these electrodes depending on the details of the medium in which they were redox cycled. For the in situ study of pseudocathodic processes involving cation transport, positively charged luminescent probes such as $Ru(bpy)_3^{2+}$ (bpy = 2,2'-bipyridyl) and acridine orange hydrochloride were employed. It is shown that the luminescence intensity modulations in the solution phase due to anion or cation transport have different polarities for the electrode potential thus mimicking an electronic (logic) device feature. The temporal aspects of the steady-state luminescence growth were analyzed for the reduction of PP/NS thin films. Diffusion coefficients of 10^{-10}-10^{-11} cm^2/s were thus obtained and were a function of the polymer film thickness. Finally, a novel potential-scan technique combining linear sweep voltammetry with in situ fluorescence monitoring was developed for PP/NS. Only a fraction (2-40%), depending on the potential scan rate and the electrolyte cation, of the total cathodic current resulted in the ejection of the NS^- ions into the fluorometrically sampled solution phase adjacent to the PP/NS thin-film electrodes. The residual current thus is attributed to the competing influence of cation transport into the polymer phase.

Introduction

Polymer-modified electrodes (PME's) have received considerable recent attention stemming in part from their relevance to a variety of application scenarios ranging from electro- and photocatalysis to controlled drug release.[1] Thus, the mechanistic issues related to charge and mass transport in these materials, aside from being fundamentally interesting, have also practical significance. An important mode of charge transport in PME's is recognized to be electron-hopping modeled as a diffusion process with a characteristic coefficient, D_{ct}.[2,3] The electron-hopping process, however, is coupled with counterion motion because of local electroneutrality considerations. The question of whether electron hopping or counterion motion controls the net rate of charge transfer has been addressed for a few PME's of the redox type.[4-6]

Literature precedents for monitoring in situ the mass transport during redox electrochemistry of PME's have been rather limited in range and scope. A major handicap associated with electrochemical probes is that the parameter measured (current, charge, or potential) has little information content of a *chemical* nature. On the other hand, spectroscopic probes have the required sensitivity and, more important, molecular specificity. In a broader context, the importance of the development of in situ probes for the understanding and subsequent optimization of ion transport dynamics in electrochemical situations has been underlined in a recent report.[7] In the spirit that new families of techniques are clearly needed for this purpose, we describe in this paper a luminescence probe technique for the in situ study of ion transport during redox electrochemistry and discuss it within the specific framework of PME's.

This is not the first instance wherein luminescence probes have been used to study PME's. Luminescence from $Ru(bpy)_3^{2+}$ (bpy = 2,2'-bipyridyl) in Nafion-modified electrodes was studied by Buttry and Anson,[8] altnough no attempts were made by these authors to correlate the excited state quenching kinetics with charge/mass transport dynamics. Subsequent studies by Majda and Faulkner[6] illustrate the usefulness of the $Ru(bpy)_3^{2+}$ probe

* Author to whom correspondence should be addressed.

(1) Reviews are contained in: (a) Murray, R. W. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Dekker: New York, 1984; pp 191-368. (b) Murray, R. W. *Annu. Rev. Mater. Sci.* **1984**, *14*, 145. (c) Faulkner, L. R. *Chem. Eng. News* **1984**, *Feb. 27*, 28. (d) Wrighton, M. S. *Science* **1986**, *231*, 32. (e) Chidsey, C. E. D.; Murray, R. W. *Ibid.* **1986**, *231*, 25. (f) Hillman, A. R. In *Electrochemical Science and Technology of Polymers-1*; Linford, R. G., Ed.; Elsevier: London and New York, 1987; pp 241-291. (g) Murray, R. W.; Ewing, A. G.; Durst, R. A. *Anal. Chem.* **1987**, *59*, 379A.

(2) For example: (a) Dahms, H. *J. Phys. Chem.* **1968**, *72*, 362. (b) Ruff, I.; Friedrich, V. J. *Ibid.* **1971**, *75*, 3297.

(3) (a) Saveant, J.-M. *J. Electroanal. Chem.* **1986**, *201*, 211. (b) Saveant, J.-M. *Ibid.* **1986**, *238*, 1. (c) Saveant, J.-M. *J. Phys. Chem.* **1988**, *92*, 1011. (d) Andrieux, C. P.; Saveant, J.-M. *Ibid.* **1988**, *92*, 6761.

(4) (a) Ikeda, T.; Schmehl, R.; Denisevich, P.; Willman, K.; Murray, R. W. *J. Am. Chem. Soc.* **1982**, *104*, 2683. (b) Ikeda, T.; Leidner, C. R.; Murray, R. W. *J. Electroanal. Chem.* **1982**, *138*, 343.

(5) White, H. S.; Leddy, J.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 4811.

(6) (a) Majda, M.; Faulkner, L. R. *J. Electroanal. Chem.* **1982**, *137*, 149. (b) Majda, M.; Faulkner, L. R. *Ibid.* **1984**, *169*, 77.

(7) Faulkner, L. R., Ed. In Situ Characterization of Electrochemical Processes, NMAB-438-3; National Materials Advisory Board, National Research Council: Washington, DC, 1987.

(8) Buttry, D. A.; Anson, F. C. *J. Am. Chem. Soc.* **1982**, *104*, 4824.

0002-7863/91/1513-3325$02.50/0