containing a cyclopropane ring. Quenching of the intermediate at an appropriate temperature creates an alternative pathway for the reaction, leading to a regioselective synthesis of β,γ-unsaturated ketones. Efforts to extend the present approach to the other carbocycle-based conjugated dienes are currently underway.

Acknowledgment. Financial support by the National Institutes of Health (Grant GM35153) is gratefully acknowledged.

Supplementary Material Available: Experimental procedures for preparing **5** and **7**, characterization data for **8–16**, and ^1H and ^{13}C NMR spectra of **5** and **7–16** (30 pages). Ordering information is given on any current masthead page.

Enzyme-like Activity of Albumins on the Thermal Back Reaction of a Photochromic Spirobenzopyran

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Received November 27, 1991
Revised Manuscript Received March 4, 1992

Recently there has been considerable interest in studies of photochromic molecules due to their potential applications to optical memory, photostimulated phase transition, photoregulation of various physical and chemical properties of polymers such as pH, polarity, and viscosity, surface wettability, potential and permeability of membranes, and so on.[2] Although control of the photochromic reaction itself is a subject of intensive research, little attention has been paid to control of thermal reactions. However, to achieve successful design of photochromic systems the thermal reaction has to be controlled as well.

Biomolecules such as antibodies, enzymes, and albumin are among the preferable matrices for controlling the thermal reaction, since they contain specific binding sites for organic molecules. Photoregulations of proteins are reported,[3] while systematic studies of the effects of biomolecules upon the properties of photochromic molecules, in particular upon the thermal reactions, have not yet been performed. In this paper we demonstrate enzyme-like activity of serum albumins toward a 6,8-dinitro-substituted spirobenzopyran.

6,8-Dinitro-1′,3′,3′-trimethylspiro[2*H*-1-benzopyran-2,2′-indoline] (6,8-dinitro-BIPS) was used (Scheme I) as a photochromic molecule.[4] Unlike most spirobenzopyrans, 6,8-dinitro-BIPS is stable in its merocyanine form (**1**). In solution the colored merocyanine (**1**) undergoes ring closure to the spiro form (**2**) when irradiated at λ = 500–600 nm. Ring opening proceeds thermally or by UV irradiation. Albumins were chosen as proteins because of their ability to bind and interact with various molecules and ions such as fatty acids, L-tryptophan, and Ca^{2+}, to mention a few.[5]

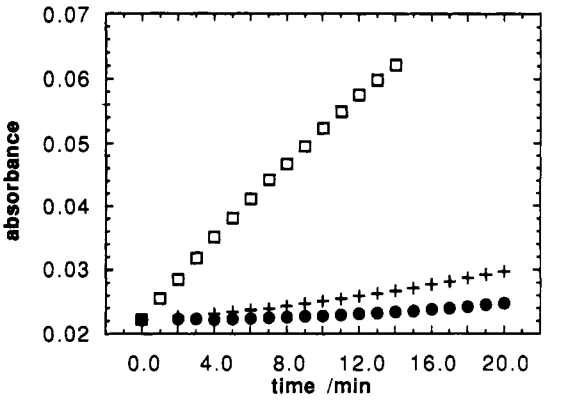
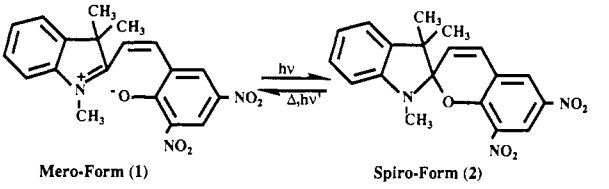


Figure 1. Thermal back reaction of the spiro form to the mero form of 6,8-dinitro-BIPS in the absence and presence of proteins: ●, without protein; □, with BSA; +, with BGG; 6,8-dinitro-BIPS, 1.82×10^{-6} M; BSA, 5.04×10^{-6} M; BGG, 4.29×10^{-6} M; monitored at 480 nm; T = 23.5 °C; path length = 5 cm.

Scheme I



The experiments were carried out as follows: the spiro form of 6,8-dinitro-BIPS was obtained by irradiating the merocyanine form in ethanol solution[6] with a Wacom super high pressure Hg lamp (500 W) for 5 min. GIF (Nikon) and Y50 (Toshiba) filters were used to select the excitation wavelength (λ = 500–600 nm). This solution was then added to phosphate-buffered saline (0.01 M PBS buffer pH 7.4, finally 2.9% (v/v) ethanol) containing human serum albumin (HSA), rabbit serum albumin (RSA), bovine serum albumin (BSA), BSA–palmitic acid (4.6 mol of palmitic acid/mol of BSA), or bovine γ globulin (BGG). Both HSA and BSA are essentially globulin and fatty acid free.[5a,7] The thermal back reaction was directly followed by measuring the absorption of the merocyanine at λ_{max} = 480 nm.

The initial temporal behavior of 6,8-dinitro-BIPS in the presence and absence of the protein is shown for BSA and BGG in Figure 1. Without albumin, slow formation of the merocyanine was observed, whereas in the presence of BSA, HSA, or RSA, the thermal back reaction was accelerated markedly. On the contrary, BGG had only a small influence, indicating that the faster formation of the merocyanine is due to the presence of albumin and not to proteins in general. After 2.5 h all molecules were converted to the merocyanine in the presence of albumin. In contrast, only 30% of the final equilibrium concentration of **1** could be obtained in the absence of albumin.

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(1) Oct 1988–Sept 1993. After September 1993 all correspondence should be sent to the permanent address of H.M.

(2) Recent reviews: (a) Krongauz, V. A. In *Photochromism, Molecules and Systems*; Duerr, H., Bouas-Laurent, H., Eds., Elsevier: Amsterdam, 1990; pp 793–820. (b) Irie, M. *Adv. Polym. Sci.* **1990**, *94*, 27–67.

(3) (a) Willner, I.; Rubin, S.; Riklin, A. *J. Am. Chem. Soc.* **1991**, *113*, 3321–3325 and references cited therein. (b) Montagnoli, G.; Nannicini, L.; Giovannitti, M. P.; Riskri, M. G. *Photochem. Photobiol.* **1978**, *27*, 43–49. (c) Karube, I.; Nakamoto, Y.; Namba, K.; Suzuki, S. *Biochim. Biophys. Acta* **1976**, *429*, 975–981.

(4) The compound was synthesized according to the following: (a) Koelsch, C. F.; Workman, W. R. *J. Am. Chem. Soc.* **1952**, *74*, 6288–6289. The melting point (280 °C) and λ_{max} = 514 nm in ethanol were consistent with the reported values; see: (b) Bertelson, R. C. In *Photochromism*; Brown, G. H., Ed.; Wiley-Interscience: New York, 1971; p 68

(5) See reviews: (a) Kragh-Hansen, U. *Dan. Med. Bull.* **1990**, *37*, 57–84. (b) Peters, T., Jr. *Adv. Protein Chem.* **1985**, *37*, 161–245.

(6) 6,8-Dinitro-BIPS is reported to be very insoluble in most organic solvents, as stated in the following: (a) Guglielmetti, R. In *Photochromism, Molecules and Systems*; Duerr, H.; Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, **1990**; p 423. (b) Hinnen, A.; Audic, C.; Gautron, R. *Bull. Soc. Chim. Fr.* **1968**, 2066–2074. We confirmed that 6,8-dinitro-BIPS was soluble in ethanol, acetonitrile, or dioxane in the range of 5×10^{-5} M; 2×10^{-6} M solutions in PBS buffer containing 2.9% (v/v) ethanol, acetonitrile, or dioxane could be prepared as well. The Lambert–Beer law held for $(0.5–3.5) \times 10^{-6}$ M, indicating no formation of aggregates.

(7) Fatty acids can bind to several binding sites of albumin: (a) Droege, J. H. M.; Janssen, L. H. M.; Wilting, J. *Biochem. J.* **1988**, *250*, 443–446. (b) Reynolds, J.; Herbert, S.; Steinhardt, J. *Biochemistry* **1968**, *7*, 1357–1361. To minimize the possibility that the observed catalytic activity of albumins is due to an unknown contamination, the catalytic activity of BSA (Sigma, BSA A-0281, less than 0.005% fatty acids, less than 1% globulin) was compared to the catalytic activity of BSA from other markers (Nacalai tesque, BSA ultrapure; Armour subdivision, BSA-CRC-7, less than 0.02% fatty acids). The results obtained from the products of Sigma and Nacalai were similar within the experimental error, while the crude product (Armour subdivision) showed less activity.

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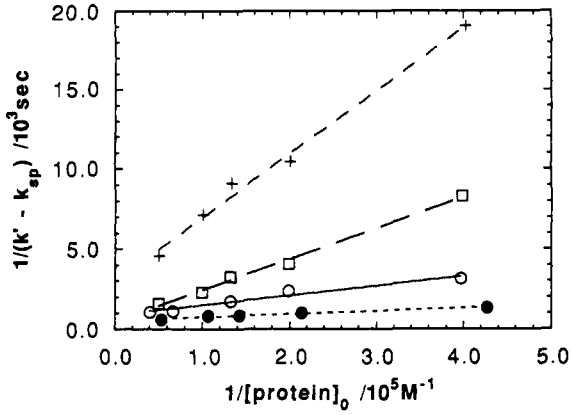

Figure 2. Plot of $1/(k - k_{sp})$ vs $1/[\text{protein}]_0$: ○, BSA; ●, BSA-palmitic acid; □, HSA; +, RSA.

To explain the acceleration of merocyanine formation by albumin the reaction was assumed to proceed through the following pathways:

$$\text{Sp} \underset{k'_{Sp}}{\overset{k_{Sp}}{\rightleftharpoons}} \text{Me} \qquad (1)$$

$$\text{Sp} + \text{Alb} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} (\text{Sp-Alb}) \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} (\text{Me-Alb}) \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} \text{Me} + \text{Alb} \qquad (2)$$

where k_{sp} = rate constant of the spontaneous thermal back reaction of the spiro form to the mero form, k'_{sp} = rate constant of the spontaneous thermal back reaction of the mero form to the spiro form (negligibly small), k_i = rate constant of each reaction step shown in eq 2, Sp = spiro form of 6,8-dinitro-BIPS, Me = mero form of 6,8-dinitro-BIPS, and Alb = albumin.

Equation 1 shows the reaction pathway in the absence of the albumin, while eq 2 represents those in its presence, provided that enzyme-like behavior is considered. In our model we assume as the first approximation that the spiro form is combined only at one binding site with high affinity. It may be called the "catalytic" binding site. To avoid the binding of 6,8-dinitro-BIPS to other sites with low affinities, the albumin was used in excess.

According to the above model, formation of the "spiro-form-albumin" complex is described by the Michaelis constant K_M; k_{+2} is just the catalytic constant k_{cat}. An apparent first-order rate constant k', describing the experimentally observed initial rate constant in the presence of albumin, can be expressed as

$$\frac{1}{k' - k_{sp}} = \frac{K_m}{(k_{cat} - k_{sp})} \frac{1}{[\text{albumin}]_0} + \frac{1}{(k_{cat} - k_{sp})} \qquad (3)$$

assuming that [albumin] = $[\text{albumin}]_0$ (=initial concentration), which is justified for albumin in excess. k_{sp} was obtained from the thermal back reaction in the absence of protein to be k_{sp} = $(1.59 \pm 0.05) \times 10^{-5}$ s^{-1} (mean value of 21 experiments) at T = (23 ± 0.5) °C. The plots according to eq 3 are shown for BSA, BSA-palmitic acid, RSA, and HSA in Figure 2.[8] The linearity of the plots indicates that the enzymatic reaction mechanism is applicable to the present systems and that eq 3 is effective. The values of K_M and k_{cat} were calculated from the slope and intercept, respectively. K_M was on the order of 10^{-5} M (BSA, HSA, RSA) and 10^{-6} M (BSA-palmitic acid). The ratio of k_{cat}/k_{sp} was 190 (BSA), 130 (HSA), 22 (RSA), or 125 (BSA-palmitic acid). The decrease of K_M while k_{cat} is nearly unchanged indicates positive cooperation of the palmitic acid. Competitive inhibition is quite unlikely because of the large structural differences between 6,8-dinitro-BIPS and fatty acids. It is worth noting that the thermal back reaction to the merocyanine is enhanced by 2 orders of magnitude for the BSA and HSA systems.

In a few cases enzyme-like activity of albumin has been reported, mainly for hydrolysis of esters and amides.[5b,9] These interactions with different substances seem to originate in the conformational fluctuations of albumin, giving it the possibility of accepting various ligands.[5a] Therefore it is likely that albumin also interacts with spiro benzopyrans.[10]

To verify the enzyme-like activity of the albumins, temperature dependences of k_{sp} and k_{cat} were measured between 10 and 35 °C. The activation energy E_a for 6,8-dintiro-BIPS itself is about 60-80 kJ/mol, which is in good agreement with the data for substituted spiro benzopyrans.[11] In the presence of BSA, E_a decreases significantly by a factor of 1.5-2.[12]

Whether the interaction is "active", involving special amino acids of the protein, or "passive", providing a suitable microenvironment for the reaction, is being examined in our group.[13]

This paper shows enzyme-like behavior of albumins toward a photochromic molecule, making this biomolecule an interesting candidate for controlling molecular systems and devices. Furthermore, the present combination of albumins and 6,8-dinitro-BIPS supports our assumption that each protein being able to bind substances can in principle act as an enzyme if a suitable substrate is chosen.[14] Further studies on the influence of albumin on the thermal back reaction and the photochromic reactions themselves are being conducted for various photochromic molecules in our laboratory.

(8) The observed initial rate constants k' were obtained as mean values from three experiments. The standard deviations of k' were less than 10%, typically 5%.

(9) Kurono, Y.; Sugiura, H.; Ikeda, K. *Chem. Pharm. Bull.* **1985**, *33*, 3966-3971 and references cited therein.

(10) An indication that spiro pyrans can be bound to BSA is given for 1'-(β-carboxyethyl)-3',3'-dimethyl-6-nitrospiro[2*H*-1-benzopyran-2,2'-indoline], but no enzyme-like behavior is mentioned: Rhee, K. W.; Gabriel, D. A.; Johnson, C. S., Jr. *J. Phys. Chem.* **1985**, *89*, 3193-3195.

(11) Bertelson, R. C. In *Photochromism*; Brown, G. H., Ed.; Wiley-Interscience: New York, 1971; p 176-189.

(12) The Arrhenius plot and detailed discussion will be published elsewhere.

(13) Preliminary results show that in solvents like dioxane, ethanol, or acetonitrile that are less polar than PBS buffer (2.9% (v/v) ethanol) the thermal reaction of the spiro form to the mero form is not accelerated. Therefore it is unlikely that acceleration caused by albumins is due to a "passive" interaction.

(14) The most famous examples are the catalytic antibodies; see, for example, recent reviews: (a) Schultz, P. G.; Lerner, A. R.; Bencovic, S. J. *Chem. Eng. News* **1990**, *68*, 26-40. (b) Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *10*, 1283.

Basic Character of Rare Earth Metal Alkoxides. Utilization in Catalytic C-C Bond-Forming Reactions and Catalytic Asymmetric Nitroaldol Reactions

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Received October 21, 1991

In a recent paper, we reported that $Zr(O\text{-}t\text{-}Bu)_4$ was an efficient and convenient basic reagent in organic synthesis.[1] However, all reactions examined were performed with stoichiometric quantities of the reagent. We envisioned that rare earth metal alkoxides would be stronger bases than group 4 metal alkoxides due to the lower ionization potential (ca. 5.4-6.4 eV) and the lower electronegativity (1.1-1.3) of rare earth elements;[2] thus, the catalytic use of rare earth metal alkoxides in organic synthesis was expected.[3] Although a variety of rare earth metal alkoxides

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(1) Sasai, H.; Kirio, Y.; Shibasaki, M. *J. Org. Chem.* **1990**, *55*, 5306.
(2) Dean, J. A., Ed. *Lange's Handbook of Chemistry*; McGraw-Hill, 1972.

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