Method 2: A solution of nBu$_4$NBr (7.85 g, 24.36 mmol) and $SOBr_2$ (8.3 mL, 105.56 mmol) in MeCN (10 mL) was added dropwise to a stirred suspension of $Na_8H[PW_9O_{34}]\cdot 7H_2O$ (20.81 g, 8.12 mmol) and K_2CO_3 (8.00 g, 57.88 mmol) in MeCN (200 mL) at $-70\,^{\circ}C$. After 1 h, the solution was filtered and the volatiles removed under reduced pressure. The resulting solid was redissolved in MeCN (40 mL) and filtered. Removal of the solvent and trituration with diethyl ether gave an orange powder (26.13 g). IR: $\tilde{\nu}$ = 1064 (s), 1022 (w), 998 (w), 989 (w), 974 (s), 928 (s), 868 (m), 787 (s, br), 737 (w), 580 (w), 519 (m) cm^{-1}.

Received: January 20, 2000
Revised: August 7, 2000 [Z14574]

[1] W. H. Knoth, *J. Am. Chem. Soc.* **1979**, *101*, 759.
[2] P. Gouzerh, A. Proust, *Chem. Rev.* **1998**, *98*, 77.
[3] W. H. Knoth, P. J. Domaille, D. C. Roe, *Inorg. Chem.* **1983**, *22*, 198.
[4] Y-J Lu, R. H. Beer, *Polyhedron* **1996**, *15*, 1667.
[5] We have shown by X-ray crystallography that this compound has the α-PW_{11} structure.
[6] Crystal data for (nBu$_4$N)$_3$-**1**$\cdot$1.56 MeCN: $C_{51.08}H_{112.62}Br_6N_{4.56}O_{28}PW_9$, M_r = 3403.9, monoclinic, space group $P2_1/n$, a = 15.0129(10), b = 25.831(2), c = 22.8859(15) Å, β = 93.452(2)°, V = 8859.0(10) Å^3, Z = 4, ρ_{calcd} = 2.552 gcm^{-3}, $Mo_{K\alpha}$ radiation, λ = 0.71073 Å, μ = 14.43 mm^{-1}, T = 160 K; of 37732 measured reflections corrected for absorption, 14773 were unique, R_{int} = 0.0668; R = 0.0600 ($I > 2\sigma$), R_w = 0.1345 (F^2, all data), GOF = 1.147, 968 parameters, final difference map extremes +2.38 and −1.20 e Å^{-3}. Disorder was refined for some alkyl chains and one partially occupied acetonitrile position with the aid of restraints. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-139046. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
[7] S. Liu, S. N. Shaikh, J. Zubieta, *Inorg. Chem.* **1988**, *27*, 3064.
[8] A. Mazeaud, N. Ammari, F. Robert, R. Thouvenot, *Angew. Chem.* **1996**, *108*, 2089; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1961.
[9] F. Xin, M. T. Pope, *Organometallics* **1994**, *13*, 4881.
[10] C. R. Mayer, R. Thouvenot, *J. Chem. Soc. Dalton Trans.* **1998**, 7.
[11] J. Lefebvre, F. Chauveau, P. Doppelt, C. Brevard, *J. Am. Chem. Soc.* **1981**, *103*, 4589.
[12] W. McFarlane, A. M. Noble, J. M. Whitfield, *J. Chem. Soc. (A)* **1971**, 948.
[13] P. J. Domaille, *Inorg. Synth.* **1990**, *27*, 100.
[14] R. J. Errington, R. L. Wingad, W. Clegg, R. A. Coxall, unpublished results.
[15] R. J. Errington, *Advanced Practical Inorganic and Metalorganic Chemistry*, Blackie Academic and Professional, London, **1997**.

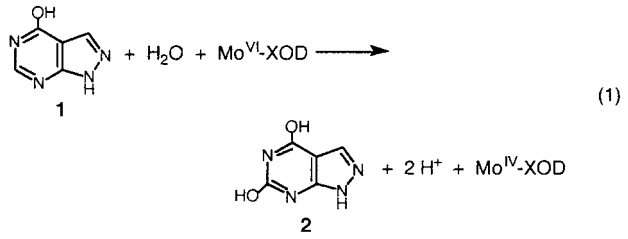
Control of Xanthine Oxidase Activity by Light**

Lin Ai Tai and Kuo Chu Hwang*

Photocontrol of enzyme activities has been a subject of active research.[1–8] Natural photoenzymes are very rare. To date, only three natural photoenzymes have been reported, namely, DNA photolyase,[9] [6–4] photoproduct lyase,[10] and protochlorophyllide reductase.[11] With their ability of transforming optical signals into chemical motion, photoenzymes might have important future applications in molecular-scale electronics in the areas of signal transformation, amplification, integration, and information storage.

Previously, photoinduced release of a light-active antigen from an antibody was reported.[12] Photochemical inhibition of nicotinic acetylcholine receptors by a photoisomerizable inhibitor was also reported.[13] Enzymes can also be chemically modified by covalently bonding a "gating molecule" to the neighborhood of the enzyme active site[3–5] or at the enzyme cofactor[6–8] to control the entry of substrates. Common gating molecules of choice are photoisomerizable olefins and photochromic compounds. Chemical modification of natural enzymes often results in partial loss of enzyme activity. Moreover, the location of the gating molecule is limited to the availability of chemically modifiable amino acids around the active site, and is quite difficult to control at will. Herein, we report a simple, novel xanthine oxidase (XOD) photoenzyme system, with allopurinol as a substrate, in which the activity can be switched on or off by light. A photoinduced intra-enzyme electron-transfer model is proposed to rationalize the photoregulation of XOD activity.

XOD is a 300 kDa homodimer protein, with each unit containing a molybdenum(VI), two Fe/S clusters, and one flavin adenine dinucleotide (FAD) moiety.[14] The Mo^{VI} ion at the active site can activate a water molecule and oxidize a C–H bond in various substrates to C–OH. After gaining two electrons from the substrate, Mo^{IV} passes two electrons sequentially, by way of the Fe/S clusters, to the FAD cofactor which then reduces molecular oxygen to generate superoxide (or hydrogen peroxide in acidic conditions).[15] As an XOD substrate, allopurinol (**1**) is oxidized and converted to alloxanthine (**2**) [Equation (1)], which is a potent, slow-



[*] K. C. Hwang, L. A. Tai
Department of Chemistry
National Tsing Hua University
Hsinchu (Taiwan)
Fax: (+886) 3-571-1082
E-mail: kchwang@mx.nthu.edu.tw

[**] The authors are grateful for financial support from the National Science Council, Taiwan (NSC 88–2113-M-007–016).

  1433-7851/00/3921-3886 $ 17.50+.50/0

binding XOD inhibitor capable of binding to the reduced form Mo^{IV}-XOD, but not to the oxidized Mo^{VI}-XOD.[16]

The enzymatic conversion of allopurinol into alloxanthine can be monitored by UV/Vis absorbance at 277 nm, the absorption maximum of alloxanthine. During the enzymatic reaction, the increase of absorption at 277 nm slowly levels off, due to the slow inhibition of XOD by alloxanthine (see Figure 1 a, trace 1). Upon exposure to the output from a high-pressure Hg lamp, the enzyme activity was recovered, as

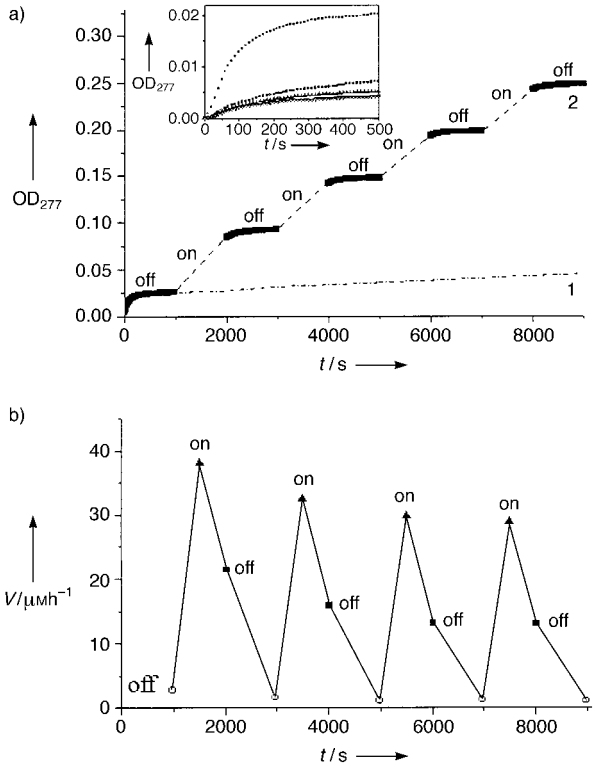


Figure 1. a) The optical density (OD) at 277 nm from a solution of allopurinol and XOD (200 μM and 0.15 μM, respectively) as a function of the reaction time. Trace 1: The solution was maintained in the dark. Trace 2: The solution was put through many dark–light cycles. In each light cycle, the solution was irradiated for 900 s (power density at the sample $\approx 0.01\ W\,cm^{-2}$; $\lambda \geq 320$ nm). Inset: The OD changes (from top to bottom) of the first, second, third, and fourth dark cycles from trace 2 as a function of the reaction time. b) The enzyme catalysis rate (V) obtained from trace 2 in (a) at different reaction times: 50 s before irradiation (open circle), during irradiation (solid triangles), and 50 s after irradiation (solid squares).

evidenced by the dramatic increase in the absorption at 277 nm (see Figure 1 a, trace 2). When the enzyme solution was removed from the light, the XOD activity was inhibited again by the alloxanthine (see Figure 1 a, the dark sections of trace 2). The XOD activity can be turned on or off by many dark–light cycles without noticeable deterioration of the enzyme activity. The absorbance change (that is, the amount of alloxanthine produced) during the "light" cycles is clearly far more than that in the "dark" cycles, indicating the dramatic photoeffect on increasing the XOD activity.

The inset of Figure 1 a shows the alloxanthine formation rate during the first 500 s of the first, second, third, and fourth dark cycles (from top to bottom). Each trace in the inset shows the typical slow-binding inhibition behavior of alloxanthine. The formation rate becomes smaller in later dark cycles, which is due to the higher concentration of alloxanthine (the inhibitor) present during later cycles. Figure 1 b shows the variation of the alloxanthine formation rates (or catalysis velocity) during the dark–light cycles. In the dark, the catalysis velocity is approximately $2\ \mu M\,h^{-1}$, before the onset of the subsequent light cycle. Upon irradiation, the catalysis velocity is increased to about $40\ \mu M\,h^{-1}$. Irradiation increases the enzyme activity by a factor of approximately 2000 %! Upon switching the light off, the catalysis velocity soon decays back to its initial low values. The data in Figure 1 clearly shows that the XOD activity can be regulated on or off photochemically.

Before irradiation, most of the XOD was inhibited by alloxanthine. To recover the enzyme activity, the inhibitor has to be released from the active site. It seems that exposure to light triggers the release of inhibitor (that is, the alloxanthine) from the XOD active site. How does light initiate the dissociation of the alloxanthine–XOD complex? It is known that alloxanthine can only bind the reduced Mo^{IV}-XOD.[16] Photoexcitation probably converts the Mo^{IV} ion into other forms, such as Mo^{V} or Mo^{VI}. The change in the electron density at the Mo ion then leads to the dissociation of alloxanthine from the active site.[17] The Mo^{VI} species does not exhibit an ESR signal, but Mo^{V} does. To examine this possibility, alloxanthine–XOD complexes were prepared by mixing XOD with excess allopurinol for 10 min. The solution was then frozen at 180 K in the presence of air, and irradiated by the output of a high-pressure Hg lamp for 3 min. Figure 2 a shows the difference spectrum obtained by subtracting the ESR spectrum after irradiation from the one obtained before irradiation. The left ESR band ($g = 2.0047$, linewidth $\Delta H_{pp} = 19.3$ Gauss) has the same g value and linewidth as that reported in the literature for FADH$^{\cdot}$.[18] The two smaller ESR bands ($g = 1.9716$, 1.9636; $\Delta H_{pp} = 8.5$, 9.0 G, respectively) resemble the "alloxanthine signal"[16, 18, 19] and are designated to the Mo^{V} species.[20] Figure 2 b shows the computer-simulated FADH$^{\cdot}$ and Mo^{V} signals.

The results in Figure 2 support the hypothesis that irradiation causes electron transfer from Mo^{IV} to the other two cofactors, Fe/S and FAD, to generate Mo^{V}, $(Fe/S)_{red}$, and FADH$^{\cdot}$ intermediate states. Subsequent electron transfer of the second electron from the Mo center to the other cofactors and release of the alloxanthine from the active site leads to recovery of free, active XOD. Therefore, the overall XOD activity is recovered by light. The ESR signal of Fe/S was not observed at the experimental conditions used ($T = 180$ K). It has been reported that the ESR signal of Fe/S has never been observed at temperatures above 42 K because of its very short relaxation time and, thus, very broad ESR linewidth.[21]

We have reported a novel XOD system in which the enzyme activity can be regulated on or off by light. This system represents a prototype of natural biological photoswitches/integrators. For example, as shown in trace 2 of Figure 1 a, the amount of alloxanthine product is continuously being integrated (or accumulated) upon stimulation by the incoming light signals. We believe that this system might have future applications as a photoswitch/integrator in molecular-scale optoelectronics.

  1433-7851/00/3921-3887 $ 17.50+.50/0

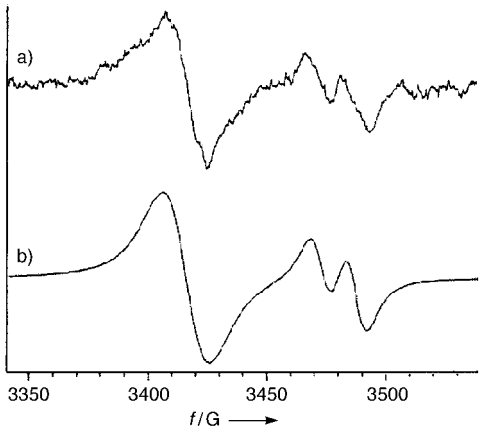


Figure 2. a) The ESR spectrum obtained by subtracting the spectrum after irradiation from the spectrum obtained before irradiation for an aqueous solution of XOD (20 μM) in the presence of excess allopurinol in air. The enzyme solution was frozen at 180 K, and the first spectrum was recorded. Then the frozen enzyme solution was irradiated (power density at the sample $\approx 0.13\ W\,cm^{-2}$) for 3 min, and the second spectrum was obtained. The microwave power was 10 mW, the modulation was 5 Gauss, and the frequency was 9.5819 GHz. b) Computer-simulated spectra of the FADH$^{\bullet}$ ($g = 2.0047$, $\Delta H_{pp} = 19.3$ G) and Mo^{V} ($g = 1.9716, 1.9636$; $\Delta H_{pp} = 8.5, 9.0$ G) signals.

Experimental Section

XOD (0.08 unit mg^{-1}, Sigma) and allopurinol (TCI) were used as received. The enzyme solution was adjusted to pH 7.5 in Na_2HPO_4 buffer (0.1M). The pH value and buffer concentration are the same in all experiments. The concentration of XOD was determined by the its absorption at 450 nm, with the absorption coefficient $\varepsilon_{450} = 37\,800\,M^{-1}\,cm^{-1}$.[22] In general, allopurinol (2 mM) in NaOH solution (0.01N) was prepared first, then aliquots of the solution were added to the enzyme solution to reach the required final concentration. The enzyme catalysis rate was monitored at the absorption maximum of the product, 277 nm for alloxanthine.

All experiments were performed in the presence of air. The light source is the output from a high-pressure Hg lamp (Oriel) fitted with a $CuSO_4$ filter solution ($\lambda \geq 320$ nm). ESR spectra were taken on a Bruker EMX-12 spectrometer. A 1,1-diphenyl-2-picrylhydrazyl (DPPH) sample in a second resonator chamber was used as the reference for the *g* value measurements. The temperature was controlled by a Bruker B-VT 2000 variable temperature control unit.

Received: February 29, 2000 [Z14789]

[1] For excellent reviews of photocontrol of enzyme activities, see: a) I. Willner, S. Rubin, *Angew. Chem.* **1996**, *108*, 419–439; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 367–385; b) I. Willner, *Acc. Chem. Res.* **1997**, *30*, 347–356; c) T. P. Begley, *Acc. Chem. Res.* **1994**, *27*, 394–401, and references therein.

[2] P. R. Westmark, J. P. Kelly, B. D. Smith, *J. Am. Chem. Soc.* **1993**, *115*, 3416–3419.

[3] I. Willner, E. Zahavy, *Angew. Chem* **1994**, *106*, 594–595; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 581–583.

[4] S. Monti, G. Montagnoli, L. Nannicini, *J. Am. Chem. Soc.* **1977**, *99*, 3808–3811.

[5] S. Martinek, I. V. Berezin, *Photochem. Photobiol.* **1979**, *29*, 637–645.

[6] R. Blonder, E. Katz, I. Willner, V. Wray, A. F. Bückmann, *J. Am. Chem. Soc.* **1997**, *119*, 11747–11757.

[7] E. Zahavy, I. Willner, *J. Am. Chem. Soc.* **1996**, *118*, 12499–12514.

[8] A. Riklin, E. Katz, I. Willner, A. Stocker, A. F. Bückmann, *Nature* **1995**, *376*, 672–675.

[9] a) D. B. Sanders, O. Wiest, *J. Am. Chem. Soc.* **1999**, *121*, 5127–5134; b) D. Burdi, T. P. Begley, *J. Am. Chem. Soc.* **1991**, *113*, 7768–7770.

[10] a) S. T. Kim, K. Malhatra, C. A. Smith, J. S. Taylor, A. Sancar, *J. Biol. Chem.* **1994**, *269*, 8535–8540; b) T. Todo, H. Takamon, H. Ryo, M. Ihara, T. Matsunaga, O. Nikaido, K. Sato, T. Nomura, *Nature* **1993**, *361*, 371–374.

[11] T. P. Begley, H. Young, *J. Am. Chem. Soc.* **1989**, *111*, 3095–3096.

[12] a) M. Harada, M. Sisido, J. Hirose, M. Nakanishi, *FEBS* **1991**, *286*, 6–8; b) R. Blonder, S. Levi, G. Tao, I. Ben-Dov, I. Willner, *J. Am. Chem. Soc.* **1997**, *119*, 10467–10478.

[13] a) J. M. Nerbonne, R. E. Sheridan, L. D. Chabala, H. A. Lester, *Mol. Pharmacol.* **1983**, *23*, 344–349; b) L. D. Chabala, H. A. Lester, *Biophys. J.* **1985**, *48*, 241–246.

[14] a) R. C. Bray, *Q. Rev. Biophys.* **1988**, *21*, 299–329; b) R. Hille, *Biochem. Biophys. Acta* **1994**, *1184*, 143–169.

[15] R. Hille, *Chem. Rev.* **1996**, *96*, 2757–2816.

[16] J. W. Williams, R. C. Bray, *Biochem. J.* **1981**, *195*, 753–760.

[17] Photooxidation of alloxanthine by the excited Mo^{IV} center is also one of the possible pathways. We isolated the alloxanthine–XOD complex and split it into two portions. One portion was maintained in the dark, waiting for dissociation into alloxanthine and the free enzyme; the other portion was photolyzed to force dissociation of the complex. The UV spectra of these samples were then compared. No clear difference was observed in the absorption of the dissociated alloxanthine. In addition, irradiation of the alloxanthine–XOD complex did not show any detectable ESR signal corresponding to an alloxanthine cation radical. Therefore, it seems that majority of alloxanthine molecules leave the enzyme's active site without being oxidized. However, the current data can certainly not exclude the possibility of photooxidation of alloxanthine as a minor process.

[18] V. Massey, H. Komai, G. Palmar, G. B. Elion, *J. Biol. Chem.* **1970**, *245*, 2837–2844.

[19] T. R. Hawkes, G. N. George, R. C. Bray, *Biochem. J.* **1984**, *218*, 961–968.

[20] The ESR data shown in Figure 2 resemble very closely the FADH$^{\bullet}$ and Mo^{V} signals of Figure 6 in Ref. [18] except that the phase of the ESR spectrometer is reversed.

[21] a) R. Hille, W. R. Hagen, W. R. Dunham, *J. Biol. Chem.* **1985**, *260*, 10569–10575; b) D. J. Lowe, R. C. Bray, *Biochem. J.* **1978**, *169*, 471–479.

[22] V. Massey, R. E. Brumby, H. Komai, G. Palmer, *J. Biol. Chem.* **1969**, *244*, 1682–1691.

  1433-7851/00/3921-3888 $ 17.50+.50/0