with absorption maxima at 325, 420, and 455 nm, as well as a shoulder at 550 nm.[12] The chemical nature of the iron–sulfur center was further confirmed as the oxidized enzyme was found to be EPR silent while the fully reduced form exhibited rhombic EPR signals having g values of 2.043, 1.960, and 1.877.[11-13] Since a total of 3 electron equiv of dithionite are required to fully reduce E_3 under anaerobic conditions, the existence of an iron–sulfur center in association with a FAD cofactor in 1:1 stoichiometry is unequivocally established. The iron–sulfur center is essential for E_3 activity as the apoenzyme, prepared by treatment with mersalyl acid,[14] is devoid of any glucoseen reductase activity.

On the basis of the physical characteristics of E_3 and their similarity to other iron–sulfur flavin containing reductases,[15] the molecular mechanisms of its catalysis can now be postulated. As depicted in Scheme II, the order of electron flow is likely to start with hydride reduction of FAD by NADH. The iron–sulfur cluster, receiving electrons one at a time from the reduced flavin, then relays the reducing equivalents to its acceptor, the E_1-bound glucoseen intermediate **4**. This proposed electron-transport sequence is mechanistically sound and is consistent with E_3's role as a two-electron/one-electron switch. The participation of a one-electron-carrying iron–sulfur center in this reduction is advantageous since both electrons are dispatched from the same redox state of the prosthetic group, allowing electrons of equal energy to be delivered to the final acceptor.[16] In light of the fact that a PMP–glucoseen adduct is the proximate acceptor receiving electrons directly from an iron–sulfur center,[17] the catalytic role of E_3, in association with E_1,[18] in the biosynthesis of ascarylose clearly constitutes a unique example of biological deoxygenation.[19] Although the radical nature of this C-3 deoxygenation process is reminiscent of the well-known sugar deoxygenation catalyzed by ribonucleotide reductase, the mechanisms of these two deoxygenations are fundamentally distinct.[20]

Acknowledgment. We thank Ms. Kim Paulsen and Dr. Charles Pace for their help on anaerobic titration and Mr. Theodore Holman and Professor John Lipscomb for their assistance with EPR measurements. This work was supported by a National Institutes of Health grant (GM 35906). H.-w.L. also thanks the National Institutes of Health for a Research Career Development Award (GM 00559).

(12) (a) Orme-Johnson, W. H.; Orme-Johnson, N. R. In *Iron-Sulfur Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1982, p 67. (b) Moura, I.; Moura, J. J. G. In *The Biological Chemistry of Iron*; Dunford, H. B., Dolphin, D., Raymond, K. N., Sieker, L., Eds., D. Reidel Publishing Co.: New York, 1982; p 179. (c) Orme-Johnson, W. H.; Orme-Johnson, N. R. *Methods Enzymol.* **1978**, *53*, 259.

(13) The co-existence of a free radical signal at $g = 2.002$ in the EPR spectrum can be ascribed to the residual flavin semiquinone (Campbell, I. D.; Dwek, R. A. In *Biological Spectroscopy*; Benjamin/Cummings: Menlo Park, CA, 1984; p 179).

(14) (a) Malkin, R.; Rabinowitz, J. C. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 822. (b) Lund, J.; Woodland, M. P.; Dalton, H. *J. Biochem.* **1985**, *147*, 297.

(15) (a) Fox, B. G.; Froland, W. A.; Dege, J. E.; Lipscomb, J. D. *J. Biol. Chem.* **1989**, *264*, 10023. (b) Berhardt, F. H.; Bill, E.; Trautwein, A. X.; Twilfer, H. *Methods Enzymol.* **1988**, *161*, 281. (c) Batie, C. J.; LaHaie, E.; Ballou, D. P. *J. Biol. Chem.* **1987**, *262*, 1510.

(16) Kamin, H.; Lambeth, J. D.; Siegel, L. M. In *Flavins and Flavoproteins*; Yagi, K., Yamano, T., Eds.; University Park Press: Baltimore, MD, 1980; Vol. 6, p 341.

(17) Other structurally analogous enzymes are all members of multicomponent oxygenase systems which employ a short electron-transport chain to catalyze electron transfer from an external donor, usually NAD(P)H, to a terminal iron containing oxygenase. The fact that the final acceptor in E_3-catalyzed sugar reduction is a highly conjugated organic molecule makes E_3 a rare, if not a unique, example of this class of enzymes.

(18) (a) Shih, Y.; Yang, D.-y.; Weigel, T. M.; Liu, H.-w. *J. Am. Chem. Soc.* **1990**, *112*, 9652. (b) Weigel, T. M.; Liu, L.-d.; Liu, H.-w. *Biochemistry*, in press. (c) Weigel, T. M.; Miller, V. P.; Liu, H.-w. *Biochemistry*, in press.

(19) The possible intermediacy of a 3,4-glucoseen–PMP in the deoxygenation process has precedent since a pyridoxal phosphate stabilized aziridine radical has been suggested as the central intermediate in the reaction catalyzed by lysine 2,3-aminomutase (Song, K. B.; Frey, P. A. *J. Biol. Chem.* **1991**, *266*, 7651 and references cited therein).

(20) (a) Stubbe, J. *Annu. Rev. Biochem.* **1989**, *58*, 257. (b) Stubbe, J. *J. Biol. Chem.* **1990**, *265*, 5329. (c) Bollinger, J. M., Jr.; Edmondson, D. E.; Huynh, B. H.; Filley, J.; Norton, J. R.; Stubbe, J. *Science* **1991**, *253*, 292.

Electron-Transfer Agents in Metal-Catalyzed Dioxygen Oxidations: Effective Catalysts for the Interception and Oxidation of Carbon Radicals

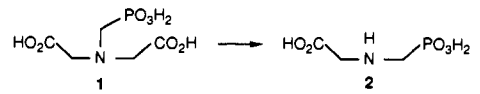
Dennis P. Riley* and Donald L. Fields

Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Received October 28, 1991

A key intermediate in metal-catalyzed autoxidations of organic substrates is often an alkyl or benzyl radical.[1] Such intermediates react with triplet molecular oxygen, forming hydroperoxy radicals whose subsequent reactions lead to such products as, e.g., aldehydes from alkyl aromatics, acids from aldehydes,[2] and alcohols and ketones from paraffins.[1] Efficient trapping of such radical intermediates with O_2 before other radical abstraction or recombination reactions occur is important for achieving high selectivity to the desired oxygenated product.

We have reported that the molecular oxygen oxidation of *N*-(phosphonomethyl)iminodiacetic acid (PMIDA), **1**, to yield *N*-(phosphonomethyl)glycine (PMG), **2**, is effectively catalyzed by cobalt(II,III)[3] and vanadium(IV,V)[4] salts in aqueous media.



1 2

This chemistry involves the formation and subsequent trapping by O_2 of an *N*-methylene carbon-centered radical, **3**, generating *N*-formyl-PMG, **4** (Scheme I). Inefficient oxygen trapping of the $NCH_2^{\bullet}$ radical, **3**, leads to the undesired *N*-methyl product, **5**, via H-atom abstraction. With V the oxidation of **1** proceeds at much faster rates, but with lower selectivities than are observed with Co. In both cases selectivity to the desired product **2** increases as O_2 pressure increases. Unfortunately, O_2 pressures over 100 atm ($\sim 1 \times 10^6$ N/m^2) are required to suppress formation of **5** in the V case.[4]

We describe in this report the first well-defined example of the use of a cocatalyst whose role is to efficiently oxidize an intermediate carbon-centered radical to the desired product, and thereby eliminate the need for high oxygen concentrations (pressure) to prevent selectivity-robbing radical processes. The introduction of a cooxidant which can intercept the *N*-methylene radical, **3**, is an attractive alternative if the cooxidant can itself be regenerated with oxygen and if it does not interfere with the primary redox processes involving O_2 oxidation of the metal complex of **1** and the subsequent metal oxidation of bound ligand. Oxidation of **3** to the iminium cation, followed by hydrolysis, would yield the desired product **2** and formaldehyde (Scheme I).

Screening studies were employed with both the cobalt and vanadium catalysts under standard experimental conditions which give 100% conversion in 200 min. For the vanadium system at 75 °C and under 200 psig of O_2, 0.017 mol of **1**/100 mL of H_2O was employed with $[VOSO_4] = 0.0085$ M ($pH_i = 1.5$). These conditions give a 50% selectivity to desired product, **2**, and ~40% selectivity to **5** in the absence of any cocatalysts.[4] For the cobalt system, screening studies were initiated using 0.088 mol of **1** in 100 mL of H_2O at 90 °C under 200 psig of O_2 with $[CoSO_4] = 0.015$ M ($pH_i = 1.5$). These conditions give a 50% selectivity to desired product, **2**, and ~40% selectivity to **5** in the absence of any cocatalysts.[4] For the cobalt system, screening studies were initiated using 0.088 mol of **1** in 100 mL of H_2O at 90 °C under 200 psig of O_2 with $[CoSO_4] = 0.015$ M ($pH_i = 1.5$). Under these conditions the cobalt system gives a 59% selectivity to **2**. Many

(1) Sheldon, R. A.; Kochi, J. K. *Metal-Catalyzed Oxidations of Organic Compounds*; Academic Press: New York, 1982; Chapter 2, pp 17-32.

(2) Riley, D. P.; Getman, D. P.; Beck, G. R.; Heintz, R. M. *J. Org. Chem.* **1987**, *52*, 287.

(3) Riley, D. P.; Fields, D. L.; Rivers, W. *J. Am. Chem. Soc.* **1991**, *113*, 3371.

(4) Riley, D. P.; Fields, D. L.; Rivers, W. *Inorg. Chem.* **1991**, *30*, 4191.

0002-7863/92/1514-1881$03.00/0

Scheme I

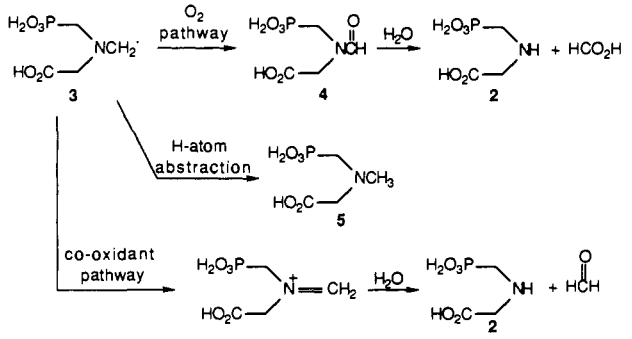
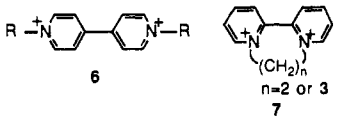


Table I

additive[a]	catalyst	selectivity to 2, %
none	Co[b]	59
none	V[c]	50
1-sulfo-9,10-anthraquinone sodium salt	Co[b]	72
2-sulfo-9,10-anthraquinone sodium salt	Co[b]	77.5
2,6-disulfo-9,10-anthraquinone disodium salt	Co[b]	73
1,5-disulfo-9,10-anthraquinone disodium salt	Co[b]	74
1-sulfo-9,10-anthraquinone sodium salt	V[c]	74
methylviologen[d] (6, R = Me)	Co[b]	78
methylviologen[d] (6, R = Me)	V[c]	96
1,1′-ethylene-2,2′-bipyridinium[d] (7, n = 2)	Co[b]	78
1,1′-ethylene-2,2′-bipyridinium[d] (7, n = 2)	V[c]	85
1,1′-trimethylene-2,2′-bipyridinium[d] (7, n = 3)	Co[b]	79
1,1′-trimethylene-2,2′-bipyridinium[d] (7, n = 3)	V[c]	88

[a] [Additive]/[MSO_4] = 0.5, where M = Co^{2+} or VO^{2+}. [b] Reaction conditions as described in text: T = 90 °C, 200 psig of O_2, reaction time = 200 min. [c] Reaction conditions as described in the text: T = 75 °C, 200 psig of O_2, reaction time = 200 min. [d] As the chloride salt.

organic oxidants were screened, and we found that two classes of oxidants, quinones and diquaternary bipyridinium salts, **6** and **7**, were effective agents for increasing the selectivity to product **2**. In addition, many different redox-active metal salts were tested



as cocatalysts under these conditions, with the result that either no effect is observed or the metal completely inhibits the reaction, as in the cases with Cu(II) and Fe(II and III) salts.

In Table I are shown some representative examples with several water-soluble quinones and bisquats. All of the additives shown in Table I have one-electron reduction potentials in the range −0.5 to −0.8 V (H_2O)[5] and are known to be efficient electron-transfer agents,[6-9] and their one-electron-reduction products react rapidly with O_2 to yield hydrogen peroxide via superoxide disproportionation.[10-12] The water-soluble quinones and bisquats shown in Table I exhibit marked selectivity-enhancing effects.[13,14] Since the V system is much more active than Co, extensive optimization studies were performed on the V system. For example, under the conditions employed in the screening study (75 °C, 200 psig of O_2), the ideal ratio of the [**6**(R=Me)] to [V] is about 4:1. With this level of added **6**, the selectivity to **2** is ~96% with only traces of the methylated product **5**. Studies with Co revealed that under a broad range of conditions the optimum ratio for the concentration of either the bisquat or quinone to [Co] is ~1.4:1. Enhanced selectivities (~93%) to **2** at low O_2 pressures (100–200 psig) result in little effect on the rate.

Studies at low O_2 pressure (50 psig, 75 °C) with V under optimized conditions ([MV]/[V] = 6) show that the level of formic acid is reduced (from 50% in the absence of electron-transfer agent, [HCO_2H] = [**2**], to ~12%) and the level of formaldehyde (from hydrolysis of the iminium cation) increases from less than 5% to ~82%. This supports our proposed mode of action for the electron-transfer cocatalysts; namely, that O_2 trapping of radical **3** is no longer required to prevent H-atom transfer to **3**. Oxidation of **3** with either the quinone or bisquat electron-transfer agent allows one to reduce O_2 pressure to much lower levels and still achieve high selectivity. Since O_2 is an efficient oxidant of the one-electron-reduction product of the additives,[10,11] O_2 remains as the ultimate oxidant in these cocatalyst systems. Each of the additives listed in Table I possesses sufficient oxidizing power to oxidize the one-electron-reduction product of an iminium cation, such as **3**.[15] Importantly, the additives show good stability in these systems; e.g., methylviologen (**6**, R=Me) is able to survive repeated recycles (10) with no loss in integrity.

The use of electron-transfer cocatalysts to intercept an intermediate in an oxygen-driven oxidation is an important concept and should have great potential for lowering the pressures required for molecular oxygen oxidations. We are continuing to pursue the mechanistic implications of these dual-component catalyst systems and are investigating their use in other O_2-catalyzed oxidations.

(5) *CRC Handbook Series in Organic Electrochemistry*; Meites, L., Zuman, P., Rupp, E., Eds.; CRC Press Inc.: West Palm Beach, FL, 1982.
(6) Methyl viologen is an electron-transfer agent in solar energy storage systems: (a) Graetzel, A. *Acc. Chem. Res.* **1981**, *14*, 376. (b) Whitten, D. G. *Acc. Chem. Res.* **1980**, *13*, 83. (c) Willner, E.; Laane, C.; Otros, J.; Calvin, M. *Adv. Chem. Ser.* **1982**, *177*, 71.
(7) Rieger, A.; Edwards, J. O. *J. Org. Chem.* **1988**, *53*, 1481.
(8) Bard, A. J.; Ledwith, A.; Shine, J. J. *Adv. Phys. Org. Chem.* **1976**, *13*, 55.
(9) Denisov, E. T.; Khudyakov, I. V. *Chem. Rev.* **1987**, *87*, 1313.
(10) The rate constant for the O_2 oxidation of the MV radical cation is ~1.2×10^7 M^{-1} s^{-1} at 12 °C: Liu, P.; Zha, Q.; Xie, C.; Li, C.; Wang, H. *Cuihua Xuebao* **1983**, *4*(2), 131.
(11) The rate constant for the O_2 oxidation of the 2,6-disulfo-9,10-anthraquinone radical anion in H_2O is 5×10^8 M^{-1} s^{-1}: Wilson, R. L. *Trans. Faraday Soc.* **1971**, *67*, 3020.
(12) (a) Evans, A. G.; Dodson, N. K.; Rees, N. H. *J. Chem. Soc., Perkin Trans. 2* **1976**, 859. (b) Evans, A. G.; Alford, R. E.; Rees, N. H. *J. Chem. Soc., Perkin Trans. 2* **1977**, 445.
(13) Fields, D. L.; Riley, D. P.; Grabiak, R. C. U.S. Patent 4952723, 1990.
(14) Fields, D. L.; Riley, D. P.; Grabiak, R. C. U.S. Patent 4937376, 1990.
(15) The $E_{1/2}$ of a representative iminium cation, 1-(4-methylbenzylidene)pyrrolidinium, is −1.36 V (NHE, H_2O). *CRC Handbook Series in Organic Electrochemistry*; Meites, L., Zuman, P., Rupp, E., Eds.; CRC Press Inc.: West Palm Beach, FL; Vol. III, p 214.

Inversion of Enzyme Enantioselectivity Mediated by the Solvent

Shinichirou Tawaki† and Alexander M. Klibanov*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received October 28, 1991

Recent evidence that enzymes can catalyze reactions in neat organic solvents has also led to the realization that enzymatic properties can be markedly altered simply by switching from one such solvent to another.[1] In particular, following our discovery[2] that enzyme enantioselectivity in nonaqueous media greatly depends on the solvent, this phenomenon has been observed, by us[3a,b] and others,[3c-g] for various asymmetric enzymatic processes. In

† On leave from Mitsui Toatsu Chemicals Co., Togo Mobara City, Japan.
(1) Klibanov, A. M. *Trends Biochem. Sci.* **1989**, *14*, 141. Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194.
(2) Sakurai, T.; Margolin, A. L.; Russell, A. J.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 7236.
(3) (a) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094. (b) Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 3166. (c) Kanerva, L. T.; et al. *Acta Chem. Scand.* **1990**, *44*, 1032. (d) Hirata, H.; Higuchi, K.; Yamashina, T. *J. Biotechnol.* **1990**, *14*, 157. (e) Parida, S.; Dordick, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 2253. (f) Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 4941. (g) Bovara, R.; Carrea, G.; Ferrara, L.; Riva, S. *Tetrahedron: Asymmetry* **1991**, *2*, 931.

0002-7863/92/1514-1882$03.00/0