Tris(picolinato)manganese(II): a chemical model for the mechanism and function of mitochondrial superoxide dismutase

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The reaction of $HO_2^{\cdot}$ with the allylic groups of lipids initiates their peroxidation and auto-oxidation, and probably represents the most serious biological hazard of $O_2^{\cdot-}$-derived species. The presence of tris(picolinato)manganese(II) [$Mn^{II}(PA)_2(PAH)(H_2O)$], a model complex for mitochondrial superoxide dismutase, (i) efficiently catalyzes the disproportionation of $O_2^{\cdot-}$, (ii) precludes the formation $HO_2^{\cdot}$, and thereby (iii) prevents hydrogen abstraction from allylic and thiol groups. Such protection demonstrates that a primary function of superoxide dismutase is to block the formation of $HO_2^{\cdot}$, which is the obligatory intermediate for the nonenzymatic proton-induced disproportionation process. This requires that the primary step for the enzyme-$O_2^{\cdot-}$ reaction be kinetically favored and dominant relative to the protonation reaction ($HA + O_2^{\cdot-}$).

Tris(picolinato)manganese(II) *Superoxide dismutase* *Model matrix* *Reaction mechanism* *Lipid peroxidation* *$HO_2^{\cdot}$ formation*

1. INTRODUCTION

The bio-generation of superoxide ion ($O_2^{\cdot-}$, from reduction of dioxygen in aerobic systems) appears to have led to the evolution of a family of metalloproteins, the superoxide dismutases (SOD), that catalyze its disproportionation and thereby protect the organism from $O_2^{\cdot-}$ toxicity [1–4]. These proteins remove $O_2^{\cdot-}$ and limit its concentration to less than 10^{-6} M (the uncatalyzed proton-induced disproportionation of $O_2^{\cdot-}$ at pH 7 in water has an observed second-order rate constant of $10^6\ M^{-1}\cdot s^{-1}$, which limits the concentration of $O_2^{\cdot-}$ to about 10^{-4} M) [5]. The catalytic mechanisms for several manganese SOD proteins, which have been isolated from prokaryotes [6,7] and from the mitochondria of eukaryotes [8–10], have been studied by pulse radiolysis [11,12].

The absence of chemically authenticated cytotoxic reactions for $O_2^{\cdot-}$ [13,14] and an enzymatic effect of only 2 orders of magnitude (with respect to lowering the $O_2^{\cdot-}$ concentration) have prompted us to seek evidence for a more unique protective function for SOD enzymes. Here, we report that the tris(picolinato)manganese(II) complex [$Mn^{II}(PA)_2(PAH)(H_2O)$] [The $Mn^{II}(PA)_2(PAH)(H_2O)$ complex was synthesized from the combination of picolinic acid (PAH) and $Mn^{II}(DMU)_6(ClO_4)_2$ or $Mn^{II}(OAc)_2(H_2O)_2$ (DMU, 1,3-dimethylurea; OAc, acetate) at a mole ratio of 3:1 in a minimum volume of methanol. The precipitated product was washed with several cold portions of methanol and ether, and dried in vacuo over P_2O_5 for 1 day. Elemental analysis – calculated for $MnC_{18}H_{15}O_7N_3$: C, 49.06; H, 3.41; O, 25.44; N, 9.54; Mn, 12.48. Found: C, 45.60; H, 3.19; O, 29.10; N, 9.29; Mn, 11.61] catalyzes the disproportionation of $O_2^{\cdot-}$ in dimethyl sulfoxide (Me_2SO), a model matrix for biomembranes where $O_2^{\cdot-}$ is formed [3]. More importantly, this model for mitochondrial SOD effectively precludes formation of $HO_2^{\cdot}$ (an obligatory intermediate for the

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uncatalyzed disproportionation of $O_2^{\cdot -}$) [5].

The perhydroxyl radical ($HO_2^{\cdot}$) abstracts hydrogen atoms from allylic groups in substrates such as 1,4-cyclohexadiene (1,4-CHD) [15] and thereby initiates their auto-oxidation and peroxidation. This hydrogen-atom abstraction represents a model for the $HO_2^{\cdot}$-initiated auto-oxidation of lipid (linoleic and arachidonic acid esters) membranes in biology.

The proton-induced disproportionation of $O_2^{\cdot -}$ by protic substrates has been evaluated in DMF and MeCN [16] and more recently in Me_2SO; the rate constant in Me_2SO is $1 \times 10^4\ M^{-1} \cdot s^{-1}$ (unpublished).

2. EXPERIMENTAL

2.1. *Equipment*

The cyclic voltammetric experiments were accomplished with a Bioanalytical Systems model CV.1B potentiostat, Brinkman electrochemical cells, and a Houston Instrument Series model 200 recorder. The working electrode was a Beckman platinum inlay (area 0.23 cm^2) and the reference an Ag/AgCl electrode adjusted with an aqueous tetramethylammonium chloride solution to a potential of 0.0 V vs SCE. The latter was contained in a glass tube with a cracked-bead closure and was placed inside a luggin capillary. All sample solutions contained 0.1 M tetraethylammonium perchlorate as a supporting electrolyte.

A Hewlett-Packard model 8457A spectrophotometer was used for UV/visible spectrophotometric measurements, and the kinetic studies made use of a Durrum model D-110 stopped-flow spectrophotometer. The data from the latter were displayed and stored on a Textronix model 564 oscilloscope.

Products of the $HO_2^{\cdot}$/1,4-CHD reaction were analyzed with a Hewlett Packard model 5880A gas chromatograph equipped with a flame-ionization detector and a 12.5 m capillary column (0.2 mm internal diameter, coated with a 0.33 μm layer of cross-linked methyl silicone).

2.2. *Reagents*

Acetonitrile (Burdick and Jackson, 'distilled in glass'), $Mn^{II}(OAc)_2 \cdot 4H_2O$ (Aldrich), picolinic

Table 1

Oxidation in Me_2SO of 1,4-CHD by $HO_2^{\cdot}$ in the presence and absence of $Mn^{II}(PA)_2(PAH)(H_2O)$[a]

Proton source (HA)	[HA] (mM)	[Mn^{II}] (mM)	[$O_2^{\cdot -}$] (mM)	[1,4-CHD] (mM)	Reaction efficiency (%)[b,c]	Product distribution (%)[c]	
						Benzene	1,3-CHD
H_2O	100	0	4.0	8.1	100	30	70
H_2O	100	0.4	4.0	8.1	19	100	0
H_2O	100	2.0	4.0	8.1	4	100	0
H_2O	100	4.0	4.0	8.1	9	100	0
H_2O	100	8.1	4.0	8.1	11	100	0
$HClO_4$	1.6	0	3.2	6.4	100	79	21
$HClO_4$	1.7	1.7	3.4	6.8	10	100	0
$HClO_4$	1.7	3.4	3.4	6.8	9	100	0
$HClO_4$	1.7	6.8	3.4	6.8	10	100	0

[a] An Me_2SO solution that contained the acid (and the Mn^{II} complex) was added to a second Me_2SO solution that contained $O_2^{\cdot -}$ and 1,4-CHD. The indicated concentrations represent the initial values after mixing

[b] 100% represents one 1,4-CHD oxidized per $O_2^{\cdot -}$

[c] Varying amounts of a proton source were dissolved in 5 ml Me_2SO and mixed with 5 ml Me_2SO stock solutions that contained $(Me_4N)O_2$ and the 1,4-CHD substrate in the absence of oxygen. The $Mn^{II}(PA)(PAH)(H_2O)$ complex was added to the solutions that contained the proton source. After cooling in an ice-water bath, 10 ml ice-cold water were added. The mixture was extracted with ether and $MgSO_4$ was added. A 0.5 μl portion was analyzed by capillary-column gas chromatography, and the product distribution and reaction efficiencies were calculated on the basis of the integrated peak areas and by adding a known amount of toluene or benzene as an internal standard

acid (Aldrich) and tetraethylammonium perchlorate (G. Frederich Smith) were used as received. The $Mn^{II}(PA)_2(PAH)(H_2O)$ complex used here was synthesized from $Mn^{II}(OAc)_2 \cdot 4H_2O$ by adding a stoichiometric amount of the ligand in a minimum volume of cold methanol. The precipitated product was washed with several portions of cold methanol and ether, and then dried in vacuo over P_2O_5 for 24 h (section 1).

3. RESULTS AND DISCUSSION

Table 1 summarizes the reactivity of $HO_2^{\cdot}$ with the allylic hydrogen atoms of 1,4-CHD. The results demonstrate that this reaction rate is competitive with the disproportionation rate for $HO_2^{\cdot}$. In dry Me_2SO, $O_2^{\cdot -}$ does not react with 1,4-CHD. The maximum yield of benzene and 1,3-CHD is obtained with $[O_2^{\cdot -}]/[HA] = 0.5$ (unpublished).

When $Mn^{II}(PA)_2(PAH)(H_2O)$ is added to $O_2^{\cdot -}$/1,4-CHD solutions that contain H_2O or $HClO_4$ as proton sources, the yield of benzene and 1,3-CHD decreases. The Mn^{II}-picolinate complex reacts with $O_2^{\cdot -}$ (before it can take up a proton from HA to form $HO_2^{\cdot}$, which disproportionates or abstracts a hydrogen atom from 1,4-CHD) to form an Mn^{III}-hydroperoxo adduct. The latter, on the basis of the spectroscopic data, reacts with a second $O_2^{\cdot -}$ to regenerate the Mn^{II} form of the catalyst and O_2. The oxygen, in turn, reacts with the $C_6H_7^{\cdot}$ radical to produce benzene as the sole product [17].

On the basis of stopped-flow spectrophotometric measurements the reaction of $O_2^{\cdot -}$ with the Mn^{II} complex (mole ratio 1 : 1) is a second-order process with a rate constant of $9.4 \pm 0.4 \times 10^5\ M^{-1} \cdot s^{-1}$, which is 2 orders of magnitude faster than the disproportionation rate constant for $HO_2^{\cdot}$ (k_d, $1 \times 10^4\ M^{-1} \cdot s^{-1}$). The reaction of $O_2^{\cdot -}$ with the $Mn^{III}(PA)_2(PAH)(OH)$ complex is also a second-order process with a rate constant of $1.3 \pm 0.4 \times 10^6\ M^{-1} \cdot s^{-1}$ in Me_2SO.

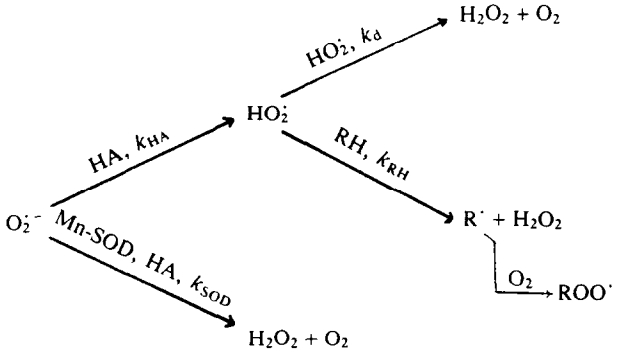
A self-consistent mechanism for the disproportionation of $O_2^{\cdot -}$ by $Mn^{II}(PA)_2(PAH)(H_2O)$ involves an initial acid-base interaction followed by electron transfer to give an Mn^{III}-(O_2H) adduct, which then oxidizes a second $O_2^{\cdot -}$.

$$Mn^{II}(PA)_2(PAH)(H_2O) + O_2^{\cdot -} \xrightarrow[HA]{k = 9.4 \times 10^5\ M^{-1} \cdot s^{-1}} Mn^{III}(PA)_2(PAH)(O_2H) + H_2O + A^- \quad (1)$$

$$Mn^{III}(PA)_2(PAH)(O_2H) + O_2^{\cdot -} \xrightarrow[HA]{k = 1 \times 10^6\ M^{-1} \cdot s^{-1}} Mn^{II}(PA)_2(PAH) + H_2O_2 + O_2 + A^- \quad (2)$$

$$Mn^{III}(PA)_2(PAH)(OH) + O_2^{\cdot -} \xrightarrow{k = 1.4 \times 10^6\ M^{-1} \cdot s^{-1}} Mn^{II}(PA)_3^- + H_2O + O_2 \quad (3)$$

In summary, a likely biological function for the SOD enzymes is to remove $O_2^{\cdot -}$ and thereby preclude formation of $HO_2^{\cdot}$. Scheme 1 outlines this catalytic chemistry for the SOD enzymes and their function in the prevention of lipid peroxidation and auto-oxidation (RH represents lipid membrane material; e.g. linoleic and arachidonic acids).



Scheme 1.

The results of table 1 confirm that an SOD model complex can block the formation of $HO_2^{\cdot}$, which requires that the primary step for the SOD/$O_2^{\cdot -}$ reaction be kinetically favored and dominant relative to the protonation reaction [$k_{SOD}(SOD) \gg k_{HA}$ in scheme 1].

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REFERENCES

[1] McCord, J.M. and Fridovich, I. (1968) J. Biol. Chem. 243, 5753–5760.
[2] McCord, J.M. and Fridovich, I. (1969) J. Biol. Chem. 244, 6049–6055.
[3] Michelson, A.M., McCord, J.M. and Fridovich, I. (1977) Superoxide and Superoxide Dismutases, Academic Press, New York.
[4] Bannister, J.V. and Hill, H.A.O. (1980) Developments in Biochemistry, vol.IIA, Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase, Elsevier, Amsterdam, New York.
[5] Sawyer, D.T. and Valentine, J.S. (1981) Acc. Chem. Res. 14, 393–400.
[6] Fridovich, I. (1975) Annu. Rev. Biochem. 44, 147–159.
[7] Brock, C.J. and Walker, J.E. (1980) Biochemistry 149, 2873–2882.
[8] Weisiger, R.A. and Fridovich, I. (1973) J. Biol. Chem. 248, 3582–3592.
[9] Steinman, H.M. (1978) J. Biol. Chem. 253, 8708–8720.
[10] Marklund, S. (1980) Acta Physiol. Scand. 492, 19–23.
[11] Pick, M., Rabani, J., Yost, F. and Fridovich, I. (1974) J. Am. Chem. Soc. 96, 7329–7333.
[12] McAdam, M.E., Fox, R.A., Lavelle, F. and Fielden, E.M. (1977) Biochem. J. 165, 71–74.
[13] Fridovich, I. (1982) Acc. Chem. Res. 15, 200; Sawyer, D.T. and Valentine, J.S. (1982) Acc. Chem. Res. 15, 200.
[14] Fee, J.A. (1982) Trends Biochem. Sci. 7, 84–86, and references therein; Fee, J.A. (1980) in: Metal Ion Activation of Dioxygen (Spiro, T.G. ed.) pp.209–237, Wiley, New York.
[15] Howard, J.A. and Ingold, K.U. (1968) Can. J. Chem. 45, 785–792.
[16] Chin, D.H., Chiericato, G., Nanni, E.J. and Sawyer, D.T. (1982) J. Am. Chem. Soc. 104, 1296–1299.
[17] Roberts, J.L. jr and Sawyer, D.T. (1983) Isr. J. Chem. 23, 430–438.