Model Compounds with Superoxide Dismutase Activity: Iron Porphyrins and other Iron Complexes

BARRY HALLIWELL and ROBERT F. PASTERNACK

Department of Biochemistry, University of London King's College, Strand, London WC2R 2LS, U.K.

The enzyme superoxide dismutase (EC 1.15.1.1) is essential to aerobic life (Fridovich, 1975; Halliwell, 1978). All superoxide dismutases are metalloproteins, containing manganese, iron or copper and zinc as the prosthetic groups. However, some simple metal complexes also react with the superoxide radical (O$_2^-$); for example, complexes of Cu(II) with amino acids (Brigelius et al., 1974) and complexes of Mn(II) with pyrophosphate (Kono et al., 1976) or with quinolinol compounds (Howie et al., 1977). Complexes of Cu(II) or Mn(II) with EDTA do not react with O$_2^-$ (Halliwell, 1975).

In contrast, complexes of Fe(II) or Fe(III) with EDTA react rapidly with O$_2^-$ (Halliwell, 1975). By competition of Fe(II)-EDTA with Nitro Blue Tetrazolium for O$_2^-$ generated in a xanthine/xanthine oxidase system at pH 10.1, a rate constant of about $3 \times 10^5 \text{M}^{-1}\text{s}^{-1}$ was calculated for the reaction of Fe(II)-EDTA with O$_2^-$ at 20°C. Complexes of iron(II) or iron(III) with diethylenetriaminepenta-acetic acid also react with O$_2^-$ at pH 10.1 ($k \sim 1 \times 10^5 \text{M}^{-1}\text{s}^{-1}$ at 20°C).

Halliwell (1975) reported that haematin reacts with O$_2^-$ at pH 10, but under the conditions used this compound would have been extensively aggregated. O$_2^-$ is known to reduce iron(III) protoporphyrin IX dimethyl ester in organic solvents (Hill et al., 1974). Certain protein-porphyrin complexes interact with O$_2^-$ in aqueous solution, including oxidized cytochrome c ($k \sim 1.1 \times 10^9 \text{M}^{-1}\text{s}^{-1}$ at pH 7.2; Koppenol et al., 1976), horseradish peroxidase ($k \sim 1 \times 10^8\text{M}^{-1}\text{s}^{-1}$ at pH 5; Sawada & Yamazaki, 1973), oxyhaemoglobin and methaemoglobin ($k \sim 4 \times 10^5$ and $\sim 6 \times 10^5 \text{M}^{-1}\text{s}^{-1}$ at pH 7; Sutton et al., 1976). However, catalase does not react with O$_2^-$ at either pH 7.8 or 10.2 (Halliwell, 1973).

As part of an investigation into the relation between the structure of porphyrins and their reaction with O$_2^-$, we have studied the properties of various metal-ion complexes of the water-soluble porphyrin tetrakis-(4-N-methyl)pyridylporphine (Fig. 1). The spectral and aggregation properties of this compound are well-known (Pasternack et al., 1977). The compound itself was found not to react with O$_2^-$ in a xanthine/xanthine oxidase system at pH 10.1, nor did its complexes with Zn(II) or Cu(II). The Co(III) derivative of tetrakis-(4-N-methyl)pyridylporphine reacted slowly with O$_2^-$, but it could not be decided if the reaction was catalytic in our system. However, the iron(III)

![Fig. 1. Structure of tetrakis-(4-N-methyl)pyridylporphine iron(III)](image-url)
complex reacted rapidly with $O_2^{--}$ in a catalytic manner. The rate constant for $O_2^{--}$ dismutation was determined by competition of the iron porphyrin with Nitro Blue Tetrazolium in a xanthine/xanthine oxidase system at pH 10.1; a value of $3 \times 10^5$ M$^{-1}$ s$^{-1}$ at 20°C was obtained. The iron porphyrin had no effect on xanthine oxidase activity. Under our reaction conditions, at least 14 molecules of $O_2^{--}$ reacted with each molecule of iron porphyrin. Unless catalase was added to the reaction mixture, the $H_2O_2$ generated caused degradation of the iron porphyrin to a product with a much lower Soret intensity, probably a bile-pigment type of compound. This product also catalysed dismutation of $O_2^{--}$ ($k = 2 \times 10^6$ M$^{-1}$ s$^{-1}$). In the presence of catalase, the Soret band of the iron porphyrin decreased and broadened slightly on first exposure to xanthine/xanthine oxidase, but then re-attained its original intensity. This suggests the intermediate formation of some iron(II) porphyrin. The simplest way of representing the reaction of tetrakis-(4-N-methyl)pyridylporphine iron(III) $[Fe(III)TMpyP]$ with $O_2^{--}$ would be:

$$Fe(III)TMpyP + O_2^{--} \rightarrow Fe(II)TMpyP + O_2$$

$$Fe(II)TMpyP + O_2^{--} + 2H_2O \rightarrow Fe(III)TMpyP + H_2O_2 + 2OH^-$$

Further investigations on the reaction mechanism and on the effect of complex-formation of the porphyrin with proteins are required.


Oxidation of Thiol Compounds by Catalase and Peroxidase in the Presence of Manganese(II) and Phenols

JOHAN DE RYCKER and BARRY HALLIWELL

Department of Biochemistry, University of London King’s College, Strand, London WC2R 2LS, U.K.

Catalase breaks down $H_2O_2$ to $O_2$ and water, but in the presence of $H_2O_2$ it can also act as a peroxidedase, oxidizing compounds such as ethanol, formate and nitrite. On exposure to acid or alkaline pH values, the catalatic activity of catalase is decreased, but the peroxidatic activity is increased (Inada et al., 1961; Marklund, 1973). For example, catalase gains the ability to oxidise NADH in the presence of $Mn^{2+}$ and phenols (Caravca & May, 1964). It also catalyses conversion of 3-hydroxyanthranilate into cinnabarinate in the presence of $Mn^{2+}$ (Savage & Prinz, 1977).

Breakdown of $H_2O_2$ by catalase is inhibited by thiol compounds (Dale & Russell, 1956; Keilin & Nicholls, 1958; Olinescu & Cotae, 1973; Scheifer et al., 1976). We report here that catalase can bring about oxidation of thiol compounds in the presence of $Mn^{2+}$ and certain phenols. Horseradish peroxidase catalyses a similar reaction (Stonier & Yang, 1973), but its specificity for phenols is different from that of catalase.

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