complex reacted rapidly with $\text{O}_2^{-}$ in a catalytic manner. The rate constant for $\text{O}_2^{-}$ 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 $3 \times 10^5 \text{M}^{-1} \text{s}^{-1}$ at 20°C was obtained. The iron porphyrin had no effect on xanthine oxidase activity. Under our reaction conditions, at least 14 molecules of $\text{O}_2^{-}$ reacted with each molecule of iron porphyrin. Unless catalase was added to the reaction mixture, the $\text{H}_2\text{O}_2$ 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 $\text{O}_2^{-}$ ($k = 2 \times 10^6 \text{M}^{-1} \text{s}^{-1}$). 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 $\text{O}_2^{-}$ would be:

$$\text{Fe(III)TMPyP} + \text{O}_2^{-} \rightarrow \text{Fe(II)TMPyP} + \text{O}_2$$  (1)

$$\text{Fe(II)TMPyP} + \text{O}_2^{-} + 2\text{H}_2\text{O} \rightarrow \text{Fe(III)TMPyP} + \text{H}_2\text{O}_2 + 2\text{OH}^-$$  (2)

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


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**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 $\text{H}_2\text{O}_2$ to $\text{O}_2$ and water, but in the presence of $\text{H}_2\text{O}_2$ it can also act as a peroxidase, 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 $\text{H}_2\text{O}_2$ 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.
Table 1. Specificity of catalase for phenols in dithiothreitol oxidation in the presence of Mn$^{2+}$

Oxidation of thiol compounds was followed at 20°C by measuring O$_2$ uptake with a Hansatech oxygen electrode calibrated as described in the manufacturer's instructions. Reaction mixtures contained, in a total volume of 2ml, the following reagents at the final concentrations stated: KH$_2$PO$_4$/KOH buffer, pH5 (50mM), thiol (5mM), MnCl$_2$ (2.25mM) and 2,4-dichlorophenol or other phenol (0.1mM). Reactions were initiated by adding catalase (0.5mg). Boehringer, or Sigma type-C40 enzymes gave comparable results. When dithiothreitol was used as substrate, its oxidation could also be followed by the increase in A$_{283}$.

<table>
<thead>
<tr>
<th>Phenol present</th>
<th>Concentration (mM)</th>
<th>Rate of dithiothreitol oxidation (nmol of O$_2$ consumed/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>79</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>0.025</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>366</td>
</tr>
<tr>
<td></td>
<td>0.1 (omit MnCl$_2$)</td>
<td>0</td>
</tr>
<tr>
<td>p-Cresol</td>
<td>0.025</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>0.1 (omit MnCl$_2$)</td>
<td>0</td>
</tr>
<tr>
<td>4-Hydroxycinnamic acid</td>
<td>0.1</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>76</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>0.1</td>
<td>79</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>0.1</td>
<td>77</td>
</tr>
<tr>
<td>Thymol</td>
<td>0.1</td>
<td>73</td>
</tr>
<tr>
<td>Catechol</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>0.1</td>
<td>15</td>
</tr>
</tbody>
</table>

Incubation of reduced glutathione or dithiothreitol with bovine liver catalase caused no detectable oxidation of the thiol compounds. Addition of MnCl$_2$ caused a very slow rate of oxidation, but the simultaneous presence of 2,4-dichlorophenol induced a rapid uptake of O$_2$, especially with dithiothreitol as substrate (Table 1). Phenols in the absence of Mn$^{2+}$ did not induce any oxidation of thiols by catalase.

Most studies were carried out with dithiothreitol as substrate, because its oxidation could also be followed by the increase in A$_{283}$ [283 nm being the $\lambda$$_{\text{max}}$ for oxidized dithiothreitol (Cleland, 1964)]. It was found that 1 mol of O$_2$ was taken up per mol of dithiothreitol oxidized. Of the phenols tested, only p-cresol and 2,4-dichlorophenol were effective in promoting dithiothreitol oxidation by catalase in the presence of Mn$^{2+}$ (Table 1). In the presence of 2,4-dichlorophenol, the concentration of Mn$^{2+}$ giving the maximum rate of dithiothreitol oxidation was found to be 2.25mM. The pH optimum in phosphate buffer was 5.0 and in acetate buffer 4.5. The reason for the different pH optima in the two buffers is unknown. The rate of dithiothreitol oxidation was proportional to the amount of catalase present up to 0.5 mg per 2ml of reaction mixture (see the legend to Table 1).

Since oxidation of NADH by catalase in the presence of Mn$^{2+}$ and phenols is inhibited by superoxide dismutase (Halliwell, 1977), the effect of this enzyme on thiol oxidation was examined; 25 units of dismutase inhibited by 20% dithiothreitol oxidation by catalase in the presence of Mn$^{2+}$ and 2,4-dichlorophenol. However, addition of an equal amount of dismutase that had been previously heated at 100°C for 10 min gave the same degree of inhibition, even though the heated dismutase had no significant activity when assayed by the method of Beauchamp & Fridovich (1971). Addition of 0.1 mg of bovine serum albumin (Sigma fraction V) also produced a 20% inhibition of dithiothreitol
oxidation. Thus the inhibition by superoxide dismutase seems to be a non-specific effect of proteins and cannot be regarded as evidence for involvement of the superoxide radical in thiol oxidation by catalase.

In agreement with previous results (Stonier & Yang, 1973), we found that horseradish peroxidase catalyses a rapid oxidation of dithiothreitol in the presence of \( \text{Mn}^{2+} \) and phenols such as \( p \)-cresol, 2,4-dichlorophenol and 4-hydroxycinnamate (which does not promote thiol oxidation by catalase; see Table 1). Oxidation of thiol compounds by horseradish peroxidase was also partially inhibited by superoxide dismutase, but application of the controls used in the experiments with catalase showed that this is again a non-specific effect of proteins.

The physiological significance of thiol oxidation by catalase is difficult to assess because of the acid pH optimum and requirement for phenols. However, even if such reactions play no role \textit{in vivo}, studies of their mechanism may help to elucidate the active-site chemistry of catalase and the relationship of this enzyme to 'real' peroxidase.


Cleland, W. W. (1964) \textit{Biochemistry} \textbf{3}, 480–482


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**Modulation of Model Biomembrane Fluidity by Hydrogenation with an Amphiphilic Catalyst**

T. D. MADDEN and P. J. QUINN

\textit{Department of Biochemistry, Chelsea College, University of London, London SW3 6LX, U.K.}

The arrangement of phospholipids in a bilayer configuration represents an important structural feature of most biological membranes. The fluidity property that is observed in these membranes is determined largely by the type of hydrocarbon chains associated with the individual phospholipid molecules. The length of these acyl chains and the presence and number of unsaturated double bonds are particularly important in this respect (Chapman, 1975).

A number of different methods have been used to alter the fatty acid composition of cell membranes of living organisms and thereby study the effects of these changes on the physical properties of the membranes and their related functions (Chapman & Quinn, 1976a).

The strategy that we have devised involves hydrogenation of the unsaturated fatty acids associated with the membrane phospholipids \textit{in situ}. This has been successfully accomplished with a homogeneous transition-metal catalyst, chlorotris(triphenylphosphine)rhodium(I) (Chapman & Quinn, 1976b). We showed that complete hydrogenation of egg phosphatidylcholine (lecithin) dispersions leads to the appearance of a sharp endothermic phase transition at \( 319 \)K associated with a conformational change of the fully saturated lecithin hydrocarbon chains (Vigo \textit{et al.}, 1978).

One of the major difficulties in using conventional homogeneous catalysts of the chlorotris(triphenylphosphine)rhodium type is that once they have been introduced into the lipid bilayer they cannot be removed without disrupting the membrane struc-