On the supposed catalytic oxidation of thiol groups by catalase

In a preliminary communication published in 1952, BOERI AND BONNICHSEN[1] reported that the oxidation of thiol compounds such as cysteine, glutathione and thioglycollic acid by molecular oxygen can be catalysed by blood or liver catalase and that these thiols inhibit the catalatic decomposition of hydrogen peroxide. The reaction postulated by these workers is of special interest because it resembles somewhat the oxidation of dioxyfumaric acid catalysed by peroxidase.

As the above communication[1] was not followed by the promised detailed report "with data on spectroscopic changes of catalase in the presence of thiol groups", the reaction between catalase and cysteine was reinvestigated both manometrically and spectroscopically. The results of this study together with some considerations based upon previous work can be summarised as follows:

1. Although both liver- and blood-catalase preparations increase the rate of auto-oxidation of cysteine, the concentration of catalase required for this reaction is about 10^5 times higher than that required for the catalatic decomposition of H_2O_2 at an approximately similar rate.

2. Boiling catalase preparations decreases the rate of the reaction as well as the total oxygen uptake in so far as the denatured protein tends to adsorb cysteine and protect it from oxidation.

3. Sodium azide, which is a powerful inhibitor of catalase, does not affect the oxidation of cysteine in this reaction.

4. Sodium pyrophosphate and $\alpha\alpha'$ dipyridyl, which combine with free iron and other metals, markedly decrease the rate of oxidation of cysteine in the presence of catalase. Ethylenediaminetetraacetic acid had little effect at high (10^{-2} M) cysteine concentrations, but was inhibitory at lower (10^{-3} M) concentrations. NN′ dihydroxyethylglycine (a commercial iron chelator, Versene Fe-3 specific) markedly stimulated the oxidation of 10^{-3} M cysteine; this stimulation occurred both with catalase and a control using free iron ($FeSO_4$).

5. Catalase, in the presence of cysteine undergoing auto-oxidation, changes its characteristic absorption spectrum (625, 540, 500 mμ) to that of the catalase-peroxide compound II with bands at 568 and 535 mμ. The effect of cysteine in this respect resembles that of ascorbic acid[2]. The spectroscopic investigation of this system may be complicated by the occurrence of free H_2S which, in the presence of peroxide, intensifies the absorption band of catalase in the red and shifts it to 635 mμ.

6. Both cysteine and ascorbic acid are ineffective as hydrogen donors in the coupled oxidation reaction catalysed by catalase in the presence of H_2O_2 generated by a primary oxidation system such as notatin, glucose and oxygen[3].

7. Glutathione behaves in a similar way to cysteine but oxidizes at a much lower rate especially when it is present in a low concentration.

8. Mammalian erythrocytes contain high concentrations both of catalase and glutathione. The oxidation of glutathione in isolated corpuscles is nevertheless very slow[4], and the glutathione in arterial blood is mainly in the reduced form[5].

9. All known catalysts of cysteine auto-oxidation (such as ionic copper or iron, and haematin) undergo reversible reduction and oxidation during this process. Catalase is reduced neither by cysteine nor by the more powerful reducing agent sodium dithionite ($Na_2S_2O_4$).

These results militate against the view put forward by BOERI AND BONNICHSEN[1] and suggest that the increase in the rate of the auto-oxidation of thiol compounds in the presence of catalase is brought about not by catalase itself but by non-haematin iron and possibly some degradation products of haematin present in catalase preparations.

Furthermore, the inactivity of catalase-peroxide compound II in the catalatic destruction of hydrogen peroxide[2] and the formation of this compound during the auto-oxidation of cysteine explain the inhibitory effect of the latter upon the activity of catalase[1,6].

One of us (P. N.) is indebted to the Medical Research Council for a personal grant enabling him to take part in this investigation.

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[1] E. BOERI AND R. BONNICHSEN, *Acta Chem. Scand.*, 6 (1952) 968.
[2] B. CHANCE, *Biochem. J.*, 46 (1950) 387.
[3] D. KEILIN AND E. F. HARTREE, *Biochem. J.*, 60 (1955) 310.
[4] N. U. MELDRUM, *Biochem. J.*, 26 (1932) 817.
[5] H. MARTIN, R. RODNIGHT, H. MCILWAIN AND M. A. TRESIZE, *Nature*, 179 (1957) 419.
[6] G. CERIOTTI, L. SPANDRIO AND E. BERTI, *Biochim. Biophys. Acta*, 23 (1957) 362.

Received February 5th, 1958