Constitution and Properties of Laccase

IT was shown that laccase, which had been inactivated by cyanide under certain conditions, could be almost completely re-activated by cupric ions, whereas other metals were ineffective[1]. Further evidence will be presented here to show that copper is an essential constituent of this enzyme. Laccase was purified by the method of D. Bertrand[2,3]. At each successive stage of purification the enzyme activity was measured in the manner previously described[1], and copper and manganese were estimated by microchemical methods[4,5]. Manganese disappears after the first steps of purification; the copper content increases with the Q_{O_2} and remains proportional to activity in all fractions up to the purest preparation having Q_{O_2} 30,000 in presence of *para*phenylene diamine (Fig. 1).

In recent papers, D. Bertrand has described three important properties of laccase which will be re-examined here. It is claimed that the activity of this enzyme is much more sensitive than that of other enzymes to small variations of pH [6]. For example, with pyrogallol as substrate, the 'relative Q_{O_2}' is said to fall from 10,000 to 1,500 when the pH changes from 6·5 to 7·0. On repeating these experiments, I found that the sensitivity of laccase to pH variations is no greater than is generally the case with enzymes, and that optimum activity is observed at pH 7·4 with pyrogallol and at pH 7·0 with *para*phenylene diamine (Fig. 2).

Furthermore, D. Bertrand found that laccase, like cytochrome oxidase, has the property of oxidizing cytochrome c [7,8] and cystein[9], and this led him to believe that the two enzymes are probably closely related. However, if laccase is added to reduced cytochrome c and the mixture shaken in air, no spectroscopic change occurs. The oxidation of cytochrome c has also been studied manometrically in the way described by D. Bertrand[7,8]. Thus the oxygen uptake at 20° C. of a system consisting of laccase and 10 mgm. hydroquinone in 3·3 ml. phosphate buffer, pH 7·1, was only increased by 5–7 per cent by the addition of 0·2 ml. of 1 per cent cytochrome c. Since, however, the quantity of cytochrome c used in such experiments is at least thirty times greater than the quantity of laccase (assuming that pure laccase has a Q_{O_2} of 20,000 in the oxidation of hydroquinone), the resulting increase in oxygen
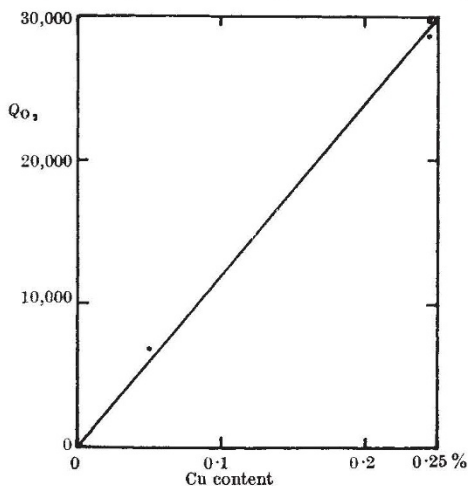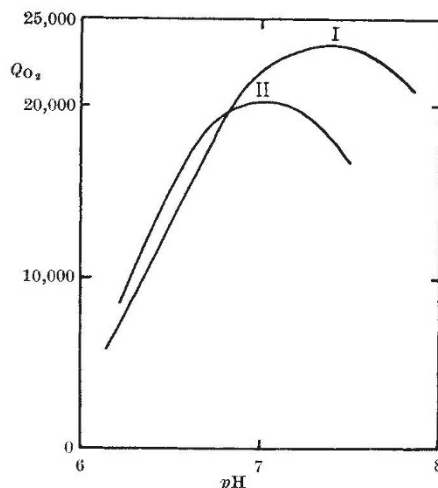
Fig. 2. pH curves of laccase activity. Curve I was obtained with pyrogallol and curve II with *para*phenylene diamine

uptake is insignificant, and there is thus no indication that cytochrome c is oxidized by laccase.

The methyl ester of cystein, as well as cystein itself, is said to be oxidized by laccase[9]. It was claimed that this oxidation has the unusual feature that the pH activity curve shows two well-marked optima at pH 6·7 and 7·45, at which the rate of oxygen uptake is 31 and 27 per cent respectively of that by hydroquinone at pH 6·35. On repeating these experiments in Barcroft manometers, it was found that cystein was not oxidized at all by laccase, even after shaking for thirty minutes. The slow rate of oxygen uptake observed with laccase and cystein methyl ester represented only 5–8 per cent of that found with laccase and *para*phenylene diamine. Moreover, a study of the effect of pH on this slow oxidation revealed not two optima, but one at 7·1, and the pH activity curve was of the usual type. As laccase does not oxidize cystein, and as its effect on the methyl ester is very small, the conclusion that cystein can be oxidized directly by laccase is not justified.

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Fig. 1. Relation between the activity of the enzyme (Q_{O_2}) and the copper content

Investigations with Radioactive Penicillin

MUCH of the published data regarding the mode of action of penicillin is necessarily concerned with secondary effects on bacteria, for example, rate of killing under different conditions, morphological changes, etc. By use of radioactive penicillin, it should be possible to find out directly something of the nature of the primary reaction between penicillin and the cell.

In a previous communication[1], we have described attempts to measure the amount of penicillin involved in the killing of bacteria, which gave no positive