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A Polarographic Study on the Catalytic Effect of the Catalase, Peroxidase and Cytochrome C Ferments on the Cathodic Reduction of Hydrogen Peroxide

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The oxygen dissolved in an aqueous solution to a concentration of about 2.5×10^{-4} *M* can be reduced to water at the dropping mercury cathode [1]. The reduction takes place in two stages. The oxygen is first reduced to hydrogen peroxide, which, in turn, is afterwards reduced to water in accordance with the following scheme [2]:

$$O_2 + 2e + 2H^+ \rightarrow H_2O_2$$
$$H_2O_2 + 2e + 2H^+ \rightarrow 2H_2O$$

This process can be followed in detail in the currentvoltage curve obtained in polarographic analysis (Fig. 1).

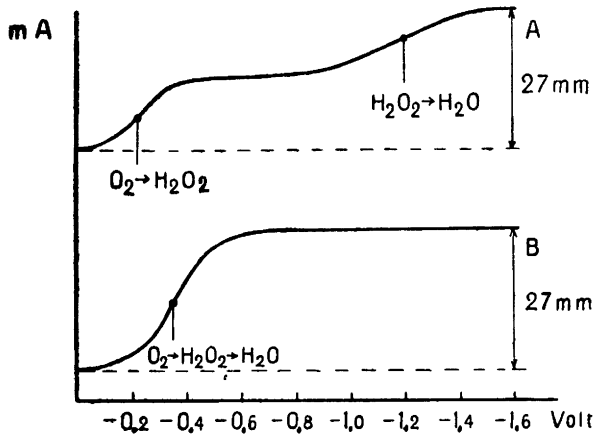

Fig. 1. Polarogram A: phosphate buffer solution pH 7.38. Polarogram B: phosphate buffer solution pH 7.38, containing 500 γ catalase/ml.

If the aqueous solution is mixed with increasing amounts of a hemin proteid, the cathodal reduction of the hydrogen peroxide will take place at a more positive potential. If the hemin proteid percentage is sufficiently increased, the two reduction stages will coincide in the polarogram, as shown by Fig. 1. This activation of the hydrogen peroxide molecule in the presence of hemoglobin and hematin has been studied by Brdicka and Tropp [3]. They found that these substances had the stated effect, which was not the case with porphyrin. The cobalt, nickel and manganese salts of hematoporphyrin were also tested [4]. These salts, however, had not the same catalytic effect as the ferric salt hematin. Brdicka and Tropp try to explain the catalytic effect by the powerful paramagnetic action which the iron in porphyrin position exerts on the likewise paramagnetic hydrogen peroxide. In a comparison between equimolar solutions of hemoglobin and hematin, they found that the hemoglobin had four times as great catalytic effect as the hemin — a difference which they supposed might be due to traces of catalase contained in the hemoglobin solution.

In order to investigate this matter, the present writer made a polarographic analysis of the conditions in the cathodic reduction of hydrogen peroxide in the presence of small amounts of pure catalase, peroxidase and cytochrome c, respectively.

OWN INVESTIGATIONS

The analyses were made with the aid of a polarograph of Leybold's make (no. 1353).

As an indifferent electrolytic conductor I used throughout a $M/15$ phosphate buffer solution pH 7.38. The pure horseradish peroxidase [5] had the activity PZ = 1160, and the catalase preparation, produced from horse liver [6], had the activity Kat. f. = 50000. The investigation was carried out, broadly speaking, in the same way as that of Brdicka and Tropp [3]. That is to say, a series of polarographic analyses were made on solutions with successive falling content of the respective hemin proteid until the concentration was reached where no catalytic affect was any longer obtained. The lowest concentration of the respective hemin proteid at which a catalytic effect could still be observed is shown in Table 1.

DISCUSSION

A comparison between the results obtained by Brdicka and Tropp and those found in the present investigation indicates that the content of hemin proteid in the solutions was of the same order of magnitude, when their catal-

Table 1. The lowest concentration of hemin at which catalytic effect could still be observed.

	Mol. wt.	Number of hemin molecules per hemin proteid molecule	Lowest conc. for catalytic effect		
			γ/ml	mole/ml x 10^{12}	Hemin molarity (mole/litre) x 10^{7}
Hemoglobin	67,000	4	2 *	29.8	1.19
Hematin	592	1	0.08 *	135	1.35
Cytochrome c	13,000	1	4	307	3.07
Peroxidase	44,100	1	10	226	2.26
Catalase	225,000	3	10	44.4	1.33

* According to Brdicka and Tropp.

ytic effect on the reduction of the hydrogen peroxide at the dropping mercury cathode set in. When the hemin content in the solutions was computed it was found that, if account was taken of experimental errors, it was equimolar. Evidently, we are not concerned here either with a specific catalase or specific peroxidase effect, but rather with a catalytic effect which may be entirely ascribed to the hemin component which, probably, has been split off from the protein at the mercury surface.

SUMMARY

The catalytic effect of hematin as well as of the hemin proteids hemoglobin, peroxidase, catalase, and cytochrome c on the cathodic reduction of hydrogen peroxide is directly proportional to the content of hemin in the solution. No potentising of this effect of specific nature in presence of these ferments, as compared whith hemoglobin, could be shown.

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