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Determination of catalase activity by means of the Clark oxygen electrode

In 1954 MAEHLY[1] pointed out that none of the then existing methods for the determination of catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) were entirely satisfactory, in the sense that they could be used as routine methods for accurate estimations of catalase activity in samples of varying purity. This situation still exists to day. The reason for this shortcoming lies in the extremely high turnover number of catalase and in the complexity of the overall kinetics of the catalytic reaction (*cf.* ref. 2). The initial rate of this reaction can only be considered constant for a very short period ($<$ 1 sec) and hence can be measured only by means of rapid mixing and recording techniques. The reaction initially follows first-order kinetics, and the velocity constant for this part of the reaction has been used as a measure of enzyme activity. However, a transition of the enzyme to a less active form takes place during the reaction, and the determination of the velocity constant must therefore be based on measurements performed before the first-order kinetics have been influenced by this transition, *i.e.* within the first minute or two. After the transition has taken place, rate changes are slow, but the rate is no longer a true measure of the enzyme activity. A reliable and at the same time generally applicable method must therefore measure the velocity constant for the initial first-order reaction.

The present experiments demonstrate that measurements of the oxygen evolved in the catalytic reaction by means of the Clark oxygen electrode may serve as a rapid and easy-to-perform assay of catalase activity in purified and in unpurified samples, provided that the membrane coating the platinum surface of the electrode is relatively impermeable to hydrogen peroxide, and that the reaction is performed in a sufficiently closed system to permit recording of oxygen concentration above that of an airsaturated solution during the short initial period, where first-order kinetics prevail.

The Clark oxygen electrode (Yellow Springs Instr. Co., Ohio, U.S.A.) was used in connection with a Micrograph recorder (Kipp, Delft, Netherlands). Pen deflections were converted to oxygen concentration on the basis of an oxygen concentration in the air-saturated medium of 0.26 mM. Linearity between values for electrode current and oxygen concentration in the range 0.26–1.26 mM was established by adding an excess of crystalline catalase to an air-saturated hydrogen peroxide solution with concentrations ranging from 0 to 2.0 mM. Except for a slow increase, corresponding to the spontaneous release of oxygen, the electrode current recorded in these hydrogen peroxide solutions before catalase addition was the same as that in the absence of hydrogen peroxide. Since hydrogen peroxide is an intermediate in the reduction of oxygen at the platinum electrode, this observation indicates that hydrogen peroxide does not reach the platinum surface. A crude estimate of the diffusion coefficient of hydrogen peroxide in the membrane was made by covering a test tube with the membrane, immersing it for 30 min in a stirred 0.1 M hydrogen peroxide solution and measuring the concentration of hydrogen peroxide inside the tube by the absorbance at 230 mμ (ref. 1). The diffusion coefficient thus found was about $3 \cdot 10^{-9}$ cm^2/sec, which, compared with the diffusion coefficient of oxygen in polyethylene ($2 \cdot 10^{-7}$–$7 \cdot 10^{-7}$ cm^2/sec; ref. 3), shows that the rate of diffusion of hydrogen peroxide must be about two orders of magnitude less than the rate of oxygen diffusion. Whether this difference

alone explains the absence of a response of the Clark electrode to hydrogen peroxide, or whether additional factors are also involved, has not been explored.

The reaction vessel (Fig. 1) was made of Perspex and constructed to accommodate the oxygen electrode as a loose-fitting stopper in order to reduce the liquid–air interphase to a minimum. Substrate addition was made through a 2-mm angular bore in the side wall in a volume of 0.05 ml or less. The volume required to fill the vessel to the level determined by the bore was 2.3 ml. The content was stirred magnetically. The above-mentioned calibration with hydrogen peroxide, as well as identity of results obtained at different initial oxygen concentrations (lowered by flushing the medium with nitrogen) for the first-order velocity constant, has shown that the equilibration of oxygen across the liquid–air interphase is sufficiently delayed to permit recording of oxygen concentrations above that of an air-saturated solution.

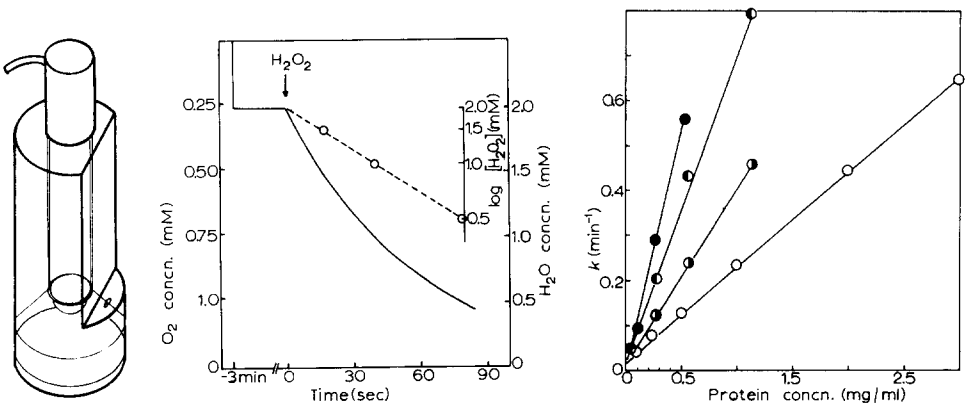

Fig. 1. Perspex vessel with Clark electrode inserted. Dimensions: height 48 mm, diameter 26 mm.

Fig. 2. Oxygen trace of H_2O_2 decomposition as catalyzed by dog erythrocytes (0.9 mg hemoglobin per ml), and semilogarithmic plot of H_2O_2 concentration calculated from O_2 concentration (broken line).

Fig. 3. Linearity of k with protein concentration in various catalase preparations. ●—●, crystalline catalase (Boehringer, Germany) in μg/ml (note the difference from the other preparations with respect to protein concentration unit); ◐—◐, lysed dog erythrocytes, in mg hemoglobin per ml; ◑—◑, intact dog erythrocytes, in mg hemoglobin per ml; ○—○, heart tissue catalase (supernatant from electron-transport particle sediment after ultrasonic treatment of mitochondrial suspension) in mg protein per ml. The figures have not been corrected for the contribution from the slow spontaneous decomposition of hydrogen peroxide.

The actual determinations of catalase activity have been performed as follows: 2.15 ml buffer and 0.1 ml enzyme sample (0°) were added to the vessel. The electrode was inserted and the magnetic stirring and the recording of electrode current was started. A sensitivity range was chosen so as to give approx. 20% of full-scale deflection for the air-saturated reaction mixture before substrate addition. After 3 min temperature equilibration, 0.05 ml hydrogen peroxide solution was added and the recording continued for approx. 90 sec. The velocity constant k was obtained graphically as shown in Fig. 2, where the slope of the broken line equals k.

With a heart-tissue catalase preparation the initial velocity ($k \cdot c_0$) was found to be directly proportional to the initial substrate concentration (c_0) at least up to 10

mM hydrogen peroxide. This is in accordance with results obtained by other "rapid" methods. With "slow" methods a gradual leveling off and eventual decline in the initial velocity is observed[2], because the increase in substrate concentration accelerates the transition to the less active form of the enzyme. Variations in k values with pH in the range 6 to 8 were only about 10%, which again demonstrated the absence of interference from the transition of the enzyme, since the rate of the transition has been shown to increase with acidity[2]. The k values were also independent of buffer concentration in the range 5 to 100 mM and identical results were obtained in Tris–HCl and in phosphate buffers.

Fig. 3 illustrates the usefulness of the method for the assay of catalase activity in samples of widely varying purity. Linearity between k values and protein concentration has been obtained with crystalline catalase, a partly purified tissue catalase preparation and erythrocyte suspensions with lysed and intact cells. In each case the linearity has been established over at least a 10-fold variation in protein concentration.

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Benzylalcohol dehydrogenase, a new alcohol dehydrogenase from Pseudomonas sp.

We have isolated and partially purified from a toluene-cultured pseudomonad an NAD-linked dehydrogenase which catalyzes the interconversion of benzyl alcohol and benzaldehyde. The enzyme, which will be referred to as "benzylalcohol dehydrogenase" (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1), is unstable, has a half-life of a few hours under conventional conditions and differs from any known alcohol dehydrogenases with respect to substrate specificity and the effectiveness of inhibitors. This report describes briefly the procedure for the purification of benzylalcohol dehydrogenase and some properties of the enzyme.

An aerobic, Gram-negative rod organism used throughout this work was isolated from soil by enrichment culture with toluene as a sole carbon source and was kindly identified as a pseudomonad by Professor T. FUJINO and Dr. Y. TAKEDA of the Research Institute for Microbial Diseases, Osaka University. The cells were grown with