THE ACTIVATION OF GULONOLACTONE OXIDASE BY HYDROGEN PEROXIDE AND BY O_2 AT HIGH PRESSURES

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It was recently reported that hydrogen peroxide activates gulonolactone oxidase to higher levels of activity than those obtained in the presence of one atmosphere of O_2 (Feinberg and Caputto, 1960). Subsequently, during the fractionation of gulonolactone oxidase, fractions were obtained which could not be activated by hydrogen peroxide and, furthermore, these fractions were also devoid of catalase. In no case was it possible to obtain in the catalase-free fraction more than 4% of the total activity of the solubilized microsomes but these experiments appear to warrant the conclusion that activation of gulonolactone oxidase by H_2O_2 requires the presence of a separable catalase.

The observation that the activity in the presence of the H_2O_2-catalase system open to the atmosphere is several times higher than when the gas phase is 100% O_2, suggested at first that the activating agent could be an intermediate in the process of releasing O_2 rather than O_2 itself. However, doubt about this hypothesis arose after observing that the measurements of Isherwood, Mapson and Chen (1960) demonstrated a rather low affinity of O_2 for rat liver gulonolactone oxidase. Subsequently the idea was discarded when we observed, unexpectedly, that this enzyme was activated to a maximum after being exposed for 10 minutes

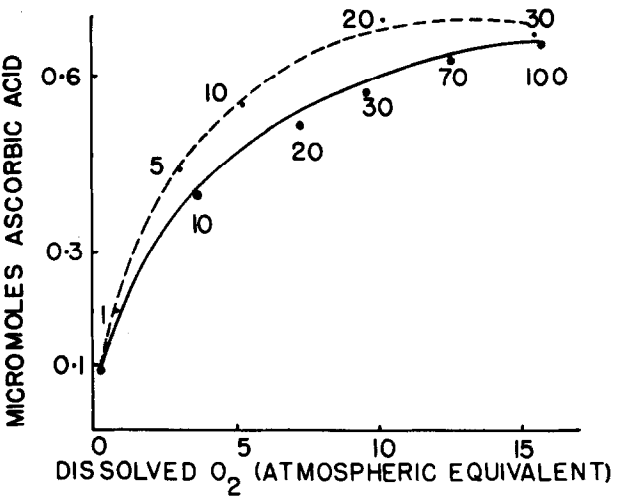

Fig. 1. Activation of gulonolactone oxidase by O_2 under pressure (- - -) and by H_2O_2 in the presence of catalase (———). Numbers above broken line are the atmospheric pressures of pure O_2 to which the solutions were subjected. Numbers below continuous line are the μmoles of H_2O_2 added. Incubation system: rat liver microsome suspension 0.1 ml; L-gulonolactone, 10 μmoles; phosphate buffer, pH 7.5, to a final volume of 1 ml; incubation period, 15 minutes; temperature, 25°C. The incubations with H_2O_2 were done in test tubes open to the atmosphere; the incubations with pressurized O_2 were done in 5 ml beakers inside a specially devised chamber; the substrate was introduced into the system from a separate container by shaking after 2 minutes of gassing and 10 minutes of equilibration.

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to 20-30 atmospheres of O_2, and that the oxygen concentrations developed in the enzyme solution by the action of catalase on the various amounts of H_2O_2 used were comparable to those obtained with the above mentioned O_2 pressures. Fig. 1 shows that the equal O_2 concentrations obtained by either solubilization of gaseous O_2 or through decomposition of H_2O_2 by catalase, the activations of gulonolactone oxidase are approximately equivalent.

The determinations of O_2 in the supersaturated solutions were made by polarographic methods. These determinations were complicated by the inherent instability of these solutions, but by employing the precaution described below, they appear to be accurate enough to establish the points reported in this communication.

The dropping mercury electrode was used either directly in the supersaturated solution or in a solution which had been diluted with oxygen-free buffer after the H_2O_2 had been destroyed by catalase (the enzyme preparations used destroyed the maximum quantity of H_2O_2 added in any experiment in less than 15 seconds). The main objection to the direct method is that the stirring caused by oxygen bubbles formed in the solution could disturb the natural diffusion rate at the mercury drop and give abnormally high current readings. The objection to the method using dilution is that some gaseous oxygen might redissolve in the oxygen-free buffer to give high values. These objections apparently are not serious because either shaking the supersaturated solution for 10 seconds just prior to dilution, or bubbling gaseous O_2 for 5 seconds, gave results that corresponded to an oxygen concentration caused by one atmosphere of O_2 pressure or less. Moreover, the values obtained by both methods were approximately the same, indicating that the bubbling in these cases had small effect on the electrode. A few attempts were made using the Clark oxygen electrode. This consisted of a platinum cathode, a silver-silver oxide reference electrode and a polyethylene membrane. This technique was discarded because it was necessary to shake the electrode in the test solution to obtain an equilibrium current value in a reasonable length of time and it was not possible to maintain a solution in a state of supersaturation with respect to O_2 during the period of shaking.

In the experiment shown in fig. 1 the enzymic activity attains a maximum at O_2 concentrations equivalent to 10 atmospheres (obtained after applying 20 atmospheres of oxygen for 10 minutes). The possibility that higher tensions of O_2 might have a destructive action on the enzyme which would conceal any activation effect was examined by keeping samples of liver microsomes from normal rats and purified preparations of solubilized gulonolactone oxidase under

air and under 30 atmospheres of O_2 for periods of up to 90 minutes at room temperature. No inactivation of the enzyme was observed by such treatment.

While there are many references to enzyme inactivation due to high O_2 pressure (Gerschman, 1959), this is, to our knowledge, the first report of an activation of an enzyme by O_2 at pressures above 1 atmosphere. It would be interesting to investigate whether or not this type of activation could be extended to other oxidases, especially those whose activities are too poor to be determined satisfactorily in air or under 1 atmosphere of O_2. These observations could initiate interest in the possible role of catalase as a local regulator of oxidases or perhaps other enzymes through the control of localized changes of O_2 tension.

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