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Experimental Studies of the Mechanism of the Peroxidase-Oxidase Reaction
in *Plant Peroxidases: Biochemistry and Physiology*
C. Obinger, U. Burner, R. Ebermann, C. Penel, H. Greppin (eds.)
University of Geneva, Geneva, 1996, p. 82 - 87.

Abstract:

We report experiments on the oscillating peroxidase-oxidase reaction, catalyzed by horseradish peroxidase., with NADH as reductant. In these experiments we study the participation of various enzyme intermediates and the roles of the modifiers 2,4-dichlorophenol (DCP) and methylene blue (MB). The experimental studies are compared with simulations of a detailed model of the reaction, the so-called BFSO scheme. According to this model about 10-20% of the enzyme should be converted into ferrous peroxidase (Per^{2+}) during an oscillation. Furthermore, the model only indirectly involves DCP and MB, since two of the reaction have rate constants which are believed to be proportional to the concentration of these two substances.

Using a diode-array spectrophotometer we have recorded the spectral changes in the interval 350 nm to 600 nm (resolution 1 nm) and the change in oxygen concentration during the oscillations. The spectral changes have been deconvoluted to show the absolute contributions of NADH and the enzyme intermediates ferric peroxidase (Per^{3+}), Per^{2+}, and compound III (coIII). The contributions of the remaining two species, compound I and compound II, could not be resolved for technical reasons. As predicted by the BFSO scheme the participation of Per^{2+} in the reaction mechanism is confirmed in these experiments. However, the amount of Per^{2+} formed during oscillations is highly dependent on the relative amounts of DCP and MB present in the reaction mixture. An increase in the ratio DCP/MB will result in an increase of the amount of Per^{2+} formed, whereas a decrease in this ratio will result in a decrease in the amount of Per^{2+} formed during an oscillation.