Mediated Electrochemistry of Horseradish Peroxidase. Catalysis and Inhibition.

Murielle Dequaire, Benoît Limoges, Jacques Moiroux and Jean-Michel Savéant

Laboratoire d'Electrochimie Moléculaire, Unité Mixte de Recherche Université - CNRS No 7591, Université de Paris 7 - Denis Diderot
2 place Jussieu, 75251 Paris Cedex 05, France.

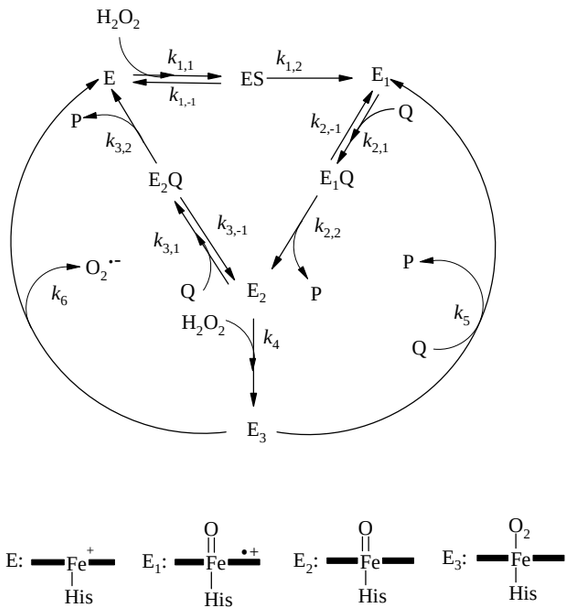
A precise determination of the complex mechanism (figure 1) of catalysis and inhibition involved in the reaction of Horseradish peroxidase with its H_2O_2 substrate and an outersphere single electron donor ($[Os(bpy)_2pyCl]^+$) cosubstrate was made possible through a systematic analysis of the cyclic voltammetric responses as a function of the scan rate and of the substrate and cosubstrate concentrations, complemented by spectrophotometric steady-state and stopped-flow experiments.

The bell-shaped calibration curve relating the electrochemical response to the concentration of H_2O_2 is qualitatively and quantitatively explained by considering the conversion of the catalytically active forms of the enzyme into the inactive oxyperoxidase in addition to the primary catalytic cycle. The ensuing analysis and data allows one to predict amperometric responses in all practical cases. From a mechanistic standpoint, conditions may however be defined which render inhibition insignificant, thus allowing an electrochemical characterization of the primary catalytic cycle.

At very low concentrations of H_2O_2, its diffusion tends to control the electrochemical response resulting in proportionality with H_2O_2 concentration instead of the square root dependence characteristic of the classical catalytic currents.

Intriguing hysteresis and trace crossings behaviors are also quantitatively explained in the framework of the same mechanism.

As a consequence of the precise dissection of the rather complex reaction mechanism into its various elementary steps, a strategy may be devised for gaining a better understanding of the mechanism and reactivity patterns of each elementary step.



P, Q: oxidized and reduced forms of the mediator

Figure 1. Scheme of the reaction mechanism of Horseradish peroxidase