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Impedance spectroscopy and conductometric biosensing for probing catalase reaction with cyanide as ligand and inhibitor

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Abstract

In this paper, we first developed a new conductometric biosensor for the detection of hydrogen peroxide. The biosensor assembly was prepared through immobilization of bovine liver catalase in a photoreticulated poly (vinyl alcohol) membrane at the surface of interdigitated microelectrodes. This biosensor was used to study the kinetic parameters of catalase-H_20_2 reaction and its inhibition by cyanide. The apparent Michaelis–Menten constants for immobilized catalase were K_{Mapp} = 81.94 ± 0.02 mM and V_{Mapp} = 13.2 µS min^{-1}, while inhibition binding constant K_i was 10.37 ± 0.04 µM. V_{maxapp} decreased by increasing cyanide concentration indicating that the inhibition process is non-competitive. In parallel, electrochemical characteristics of the catalase/PVA biomembrane and its interaction with cyanide were studied by cyclic voltammetry and impedance spectroscopy. Addition of the biomembrane onto the gold electrodes induced a significant increase of the interfacial polarization resistance R_P. On the contrary, cyanide binding resulted in a decrease of R_P due to the modification of enzyme conformation. Inhibition coefficient I_{50} calculated by this powerful label-free and substrate-free technique (24.3 µM) was in good agreement with that determined from the substrate-dependent conductometric biosensor (24.9 µM).

Keywords: cyanide, catalase, inhibition, conductometric biosensor, Impedimetric biosensor, I_{50}.