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Purification and Partial Characterization of Catalase from Chicken Erythrocytes and the Effect of Various Inhibitors on Enzyme Activity

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Abstract: Catalase plays a major role in the protection of tissues from the toxic effects of H_2O_2 and partially reduced oxygen species. A nearly 136-fold enzyme purification was obtained from chicken erythrocyte by acetone precipitation, ethanol-chloroform treatment, CM-cellulose and Sephadex G-200 chromatography. The specific activity of purified enzyme was 42,556 U/mg. The molecular weight of the native chicken erythrocyte catalase was estimated at 240 kDa by gel filtration. SDS-gel electrophoresis results indicated that chicken erythrocyte catalase consists of four apparently identical subunits, with a molecular weight of around 57.5 kDa. The optical spectrum of the purified enzyme shows a Soret band at 406 nm, which is the characteristic for the heme group. Dithionite treatment of the enzyme resulted in the reduction of enzyme. The Km of chicken erythrocyte catalase was 33 mM H_2O_2. The maximal activity of catalase was observed between pH 6.0 and 8.0. Enzyme activity was stable at temperatures between 10 and 30°C. The activity of purified catalase was inhibited by azide, cyanide, β -mercaptoethanol, dithiotreitol (DTT) and iodoacetamide.

Key Words: Chicken erythrocyte, antioxidant enzyme, purification, characterization, isolation, inhibitors

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