Abstract

Candida tropicalis yeast and bacteria *Comamonas testosteroni* have been considered to be able to metabolize phenol and utilize it as the only source of carbon and energy. In our laboratory we investigated the cytoplasmic enzymes responsible for the first and second step of phenol degradation, NADPH-dependent phenol hydroxylase of both *C. tropicalis* and *C. testosteroni* and catechol 1,2-dioxygenase of *C. tropicalis*.

The aim of our study was to isolate and partially characterize those enzymes. Phenol hydroxylase purification consisted of preparation of cytosol from *C. tropicalis* yeast by fraction centrifugation, chromatography and re-chromatography on a column of DEAE Sepharose, fractionation by precipitation of the enzyme with polyethylene glycol 6000 and gel permeation chromatography on a column of Sephacryl S-300. Extracellular phenol hydroxylase of *C. testosteroni* was purified by fraction precipitation with polyethylene glycol 6000 and by gel permeation chromatography on 4B Sepharose and Sephacryl S-300. Catechol 1,2-dioxygenase was purified using the procedure consisting of: chromatography and re-chromatography on a column of DEAE Sepharose, lyophilization of the enzyme and gel permeation chromatography on a column of Sephadex G-100.

The enzyme activity was determined by two methods: use of HPLC and/or spectrophotometrically. The purity of enzymes was analyzed with sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The molecular mass of the enzymes was determined by SDS-PAGE and gel permeation chromatography on Sephacryl S-300/Sephadex G-100. NADPH-dependent phenol hydroxylase of *C. tropicalis* consists of four identical subunits having molecular mass of 60 ± 5 kDa. pH optimum of the enzyme to catalyze phenol oxidation to catechol was determined to be pH 7.6. The kinetic parameters were also determined. Phenol hydroxylase activity is inhibited by some heavy metal ions, particularly by Cu^{2+} and Pb^{2+} ions. NADPH-dependent phenol hydroxylase of *C. testosteroni* consists also of four identical subunits; its molecular mass is 240 ± 5 kDa. The kinetic parameters were determined. Catechol 1,2-dioxygenase is a dimer having a molecular mass of 63 ± 5 kDa. pH optimum of the enzyme to form *cis,cis*-muconic acid is pH 7.7 and temperature optimum of the enzyme for this reaction is 30°C. The kinetic parameters of the enzyme were determined and sensitivity to heavy metal ions was proved. The most effective inhibition was produced by Pb^{2+} followed by Mn^{2+}, Cd^{2+} ,Fe^{2+} and Cu^{2+} ions.

Keywords: NADPD-dependent phenol hydroxylase, catechol 1,2-dioxygenase, *Candida tropicalis* yeast, *Comamonas testosteroni* bacteria, phenol, bioremediation