E303

BIOCHEMICAL CHARACTERISTICS AND CATALYTIC MECHANISM OF *PSEUDOMONAS PUTIDA* CATECHOL 2,3-DIOXYGENASE

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The catechol 2,3-dioxygenase (C23O) encoded by *Pseudomonas putida xylE* gene was overproduced in *Escherichia coli* BL21 and purified to homogeneity. The recombinant C23O was shown to depend on a metal ion in the reduced state for its optimal activity since the recombinant enzyme was highly susceptible to inactivation by air oxygen during purification or treatment with hydrogen peroxide but reactivated upon addition of ferrous sulfate in conjunction with ascorbic acid. Histidine, tyrosine and glutamic acid, the three amino acid residues conserved in extradiol-cleaving dioxygenases can serve as coordinating ligands for an iron metal. To determine whether these residues play a key function in the C23O catalysis, we carried out chemical modification of these amino acids and checked activity of the modified enzymes. The C23O activity was inactivated by treatment with DEPC, TNM and CMC, the chemical reagents targeting histidine, tyrosine and glutamic acid, respectively. These three conserved amino acids may play a role in the C23O catalysis by providing Fe^{2+}-coordinating ligands. We are currently analyzing the essential residues for the enzyme activiy by PCR-mediated mutagenesis.

E304

Degradation of dibenzothiophene (DBT) by cell-free extract of *Desulfovibrio desulfuricans* M6

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For the localization of the DBT degrading enzyme(s), the cell-free extracts (CFE) of *Desulfovibrio desulfuricans* M6 was used. The specific activity of DBT degradation was higher in CFE than in the intact cell. The activity was recovered in the soluble fraction of CFE from an ultracentrifuge, and precipitated by ammonium sulfate of 70-80% saturation. Decrease in the activity of the CFE was observed when the CFE was added by methyl viologen or carbon monoxide. The activity became unstable with time even at low temperature under anaerobic condition. The inactivation was faster when the CFE was exposed to the air.