

Crystallographic studies of chlorocatechol 1,2-dioxygenase from *Pseudomonas putida*.

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Accumulation of persistent organic pollutants is one of the most important environmental problems worldwide. Industrial activities and technological advances contribute to the spread of pollutants which are highly toxic, bioaccumulative, and resistant to physical, chemical, photolytic and biological degradation. The persistent organic pollutants are consequence of the inappropriate use of pesticides, the production of toxic synthetic compounds such as polychlorinated biphenyls and a large list of activities promote incomplete combustion of which organic matter and release a large amount of persistent aromatic hydrocarbons to the environment. Bioremediation is a biotechnological strategy that has shed light on revitalization techniques of contaminated sites. It is based on the application of microorganisms, or their enzymes, to eliminate or reduce environmental contaminants into inert substances, CO₂ and water. The oxygenases are a class of largely studied enzymes due to their catalytic properties, being chlorocatechol 1,2-dioxygenase from *Pseudomonas putida* an interesting biotechnological target for bioremediation due to its specificity to a broad spectrum of highly polluted aromatic substrates. In the present work, heterologous expression protocol has been implemented in our laboratory and purification was performed by using affinity column in chitin resin. Brown-reddish crystals of chlorocatechol 1,2-dioxygenase from *Pseudomonas putida* were obtained in presence of *polyethylene glycol (PEG)* and magnesium acetate after 10 days, by utilizing vapor diffusion techniques in sitting drops. The crystallographic structure of Pp 1,2-CCD has been solved by MR-SAD technique, using iron atoms, the enzyme cofactor, as scattering centers and the coordinates of 3-chlorocatechol 1,2-dioxygenase of *Rhodococcus opacus* as the search model. The final model, containing three molecules in the asymmetric unit, has been refined up to 3.4 Å resolution. The enzyme folds in the dimeric form ($\alpha\beta\text{Fe}^{3+}$)₂, and shows two catalytic domains composed of β -strands and a loop region, and a helical domain that pack together forming a hydrophobic channel. As being the first member of the chlorocatechol dioxygenase family of enzymes from a gram-negative bacteria to be solved, it will be possible to explore the crystallographic structure of chlorocatechol 1,2-dioxygenase from *Pseudomonas putida* in order to investigate the conformational differences that explain its mechanism of action, on a broad spectrum of substrates, and evaluate the functional features, as an important tool to validate the role of this enzyme in the bioremediation process.